



CHAPTER IV

RESULTS

1. Preparation of Low Lactose Milk-based Medical Food

1.1 Efficiency of Lactase

HPLC chromatogram of standard sugars is illustrated in Figure 3. The retention times for sugars of milk (lactose, glucose and galactose) were 6.23, 3.72 and 3.97 min. Analysis of lactose showed that the content in pasteurized low fat milk was 5.01 percent and it was inversely varied with the amount of lactase. As suggested by Lactaid Inc., lactose is reduced more than 70 percent by adding 5 drops of Lactaid in a quart of milk. Adding 10 drops or 15 drops per quart, the lactose will be reduced by more than 90 or 99.. percent, respectively. Analysis of sugars of untreated milk and of lactase-treated milk (15 drops of Lactaid per quart for 24 hr)(Figure 3) showed that major sugar in untreated milk was lactose while those in lactase-treated milk were glucose and galactose (Retention times of HPLC were 6.23 and 3.72 min, respectively). The chromatogram of lactase-treated milk had only one detectable peak because the determination on the amount of glucose and galactose was not the main objective of this investigation.

1.2 Preparation of Spray-dried Low Lactose Milk-based Medical Food

In order to get fine, dried powder of spray-dried low lactose milk-based medical food, preliminary investigation was carried out with a batch size of 946 ml (1 quart) pasteurized low fat milk.

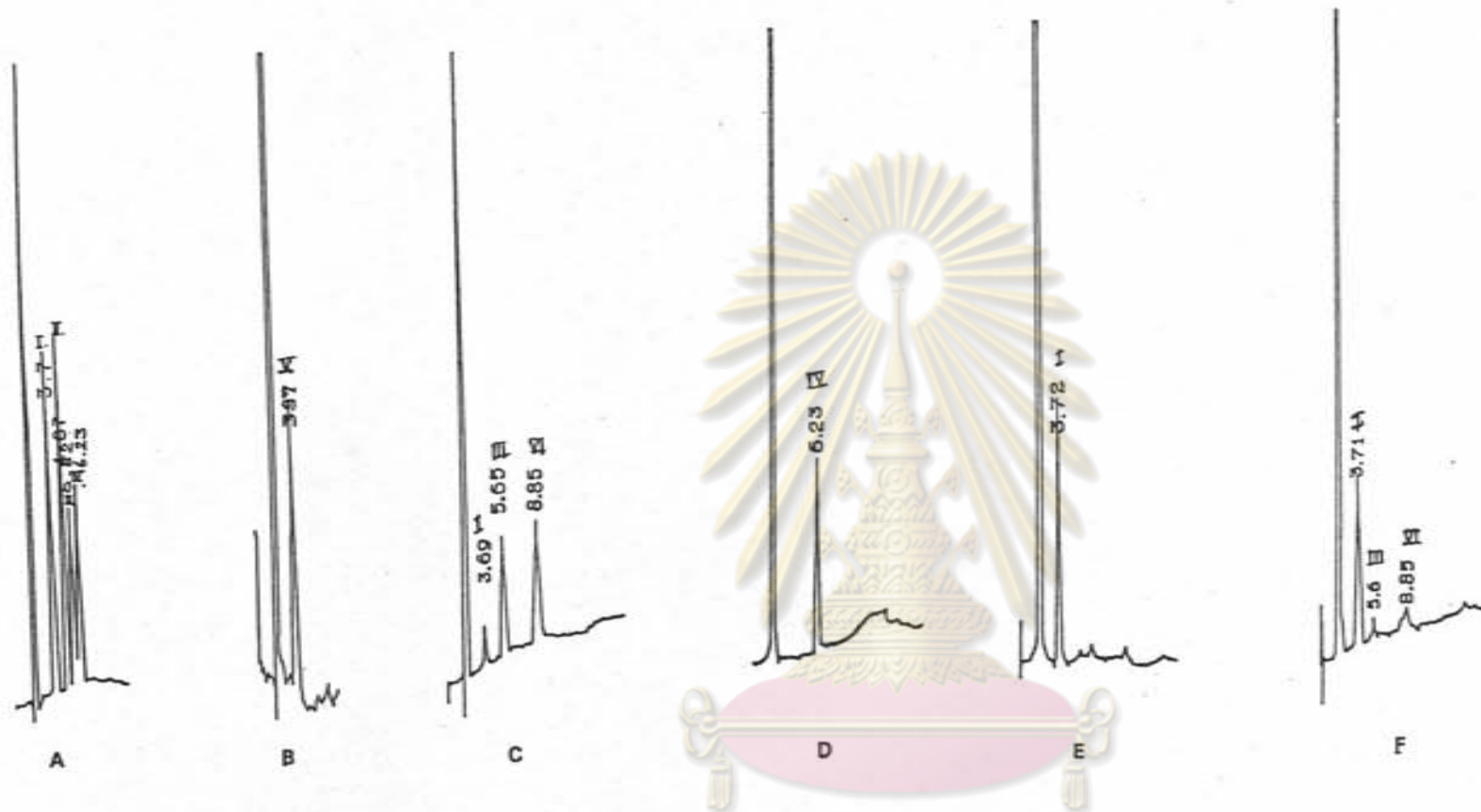


Figure 3 HPLC separation of saccharides

(peak no. I= glucose , II=sucrose , III=maltose , IV=lactose , V=galactose , VI=unidentified)

(A) standard mixture of sugars

(B) galactose standard solution

(C) maltodextrin

(D) untreated milk

(E) lactase-treated milk

(15 drops of Lactaid/ 1 quart of milk)

(F) spray-dried low lactose milk-based medical food

Running Condition: column was LC-NH₂ Supelco, Shimadzu (4.5 mm i.d.x25 cm); detector was RI detector

mobile phase was acetonitrile/H₂O 75/25 V/V , flow rate 2 ml/min.

Optimum Condition of the Spray Drying Process:

Percentages of lecithin were varied from 0.1-0.5 percent and the apparent colloidal stability was observed. It was found that 0.3 percent of lecithin was the suitable amount for spray drying milk-based emulsion.

Several variable factors such as inlet air temperature, outlet air temperature and spraying rate were varied and examined. The color, bulk density and apparent colloidal stability of spray-dried product at different drying temperature (130-80°C, 140-90°C and 150-100°C) were similar, (Table 3). Solubility Index was increased while moisture content decreased with respect to the increase of drying temperature. Therefore, the optimal processing conditions for preparation of spray-dried low lactose milk-based medical food were :

- : spraying rate 5 ml/min
 - : inlet air temperature 130°C
 - : outlet air temperature 80°C
 - : aspirator full speed
- (preconditioned with distilled water)

2. Physical Properties of Spray-dried Low Lactose Milk-based Medical Food

Bulk density, solubility index and apparent colloidal stability of the spray-dried product are shown in Table 3.

3. Chemical Compositions of Spray-dried Low Lactose Milk-based Medical Food

Proximate analysis of spray-dried product were performed. The moisture, fat, protein, carbohydrate and ash contents are presented in Table 4. Fat, protein and carbohydrate contents in 100 g product were 18.12 ± 0.04 , 16.69 ± 0.05 and 58.56 ± 0.21 g,

Table 3 Properties of Spray-dried Low Lactose Milk-based Medical Food Sprayed at Different Drying Temperature

Properties	Temperature (Inlet-Outlet)°C		
	130-80 #	140-90	150-100
Color	off-white	off-white	off-white
Bulk Density (g/ml)	0.48(0.02) *	0.46(0.02)	0.49(0.02)
Solubility Index (ml)	< 0.1	0.2 (0.08)	0.4 (0.1)
Apparent Colloidal Stability	stable	stable	stable
Moisture Content (%)	2.84±0.03	1.89(0.04)	1.01(0.02)
Yield (%) **	17.93±2.50	19.34(3.70)	15.84(2.80)

* values presented as means (S.D.) of five replica

** percentages of yield = $\frac{\text{weight of dried product (g)} \times 100}{\text{volume of sample before spray dry (ml)}}$

the best temperature for spray drying

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Table 4 Compositions of the Spray-dried Low Lactose Milk-based Medical Food

Constituents	g/100 g Sample	Cal/100 g Sample	Caloric Distribution(%)
Moisture	2.84 (0.09)	-	-
Total Solid	97.16 (0.09)	-	-
Fat	18.12 (0.04)	163.08 (0.36)	35.14 (0.08)
Protein	16.69 (0.05)	66.76 (0.20)	14.39 (0.04)
Carbohydrate	58.56 (0.21)	234.24 (0.84)	50.47 (0.18)
Ash	3.79 (0.03)	-	-
Lactose	ND*	-	-
Total	100.00	460.08	100.00

values presented as means (S.D.) of five replica

* not detectable

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respectively. The caloric distribution of this formulation was 35.14 ± 0.08 , 14.39 ± 0.04 and 50.47 ± 0.18 percent of total calories, with respect to fat, protein and carbohydrate. The non-protein calories to nitrogen ratio was 148.79 Cal per 1 g nitrogen.

Saccharide contents of spray-dried product were analyzed with HPLC method. It demonstrated that there was no lactose in this spray-dried product (Figure 3).

The chromatogram of amino acid analyzed with amino acid analyzer is shown in Figure 6, (Appendix C). The amino acid contents of spray-dried product is presented in Table 5. The essential amino acid contents of spray-dried product (Table 6) were compared with FAO/WHO amino acid reference protein (Figure 4). Amino acid scores of isoleucine, leucine, lysine, methionine with cystine, threonine, phenylalanine with tyrosine and valine were 1.31, 1.24, 0.46, 1.08, 0.94, 1.32 and 1.13, respectively. The amino acid score of lysine was the lowest; thus, lysine was considerably the limiting amino acid in this product.

4. Biological Assay

4.1 Determination of Protein Efficiency Ratio

Body weight, protein intake and PER values of ten rats in three assigned groups namely, casein reference group, low lactose group and lactose group, are presented in Table 7. Mean PER for casein reference group, low lactose group and lactose group were 2.72 ± 0.25 , 2.85 ± 0.48 and 3.07 ± 0.48 , respectively. The analysis of variance for PER of each group is shown in Table 10, (Appendix D). There was no statistically significant difference among three groups of rats ($p > 0.05$). A correction to casein, CPER, of low lactose group and lactose group were 2.62 ± 0.47 and

Table 5 Amino Acid Contents of Spray-dried Low Lactose Milk-based Medical Food

Amino Acids	mg/g Sample	mg/g Protein
Cystine	1.47	8.81
Methionine	4.83	28.94
Aspartic	11.38	68.19
Threonine	6.30	37.78
Serine	7.45	44.64
Glutamic	30.38	182.01
Glycine	2.87	17.17
Alanine	4.83	28.94
Valine	9.42	56.42
Proline	15.23	91.25
Isoleucine	8.76	52.49
Leucine	14.49	86.83
Tyrosine	6.14	36.79
Phenylalanine	7.12	42.68
Lysine	4.26	25.51
Histidine	4.01	24.04
Arginine	5.32	31.89

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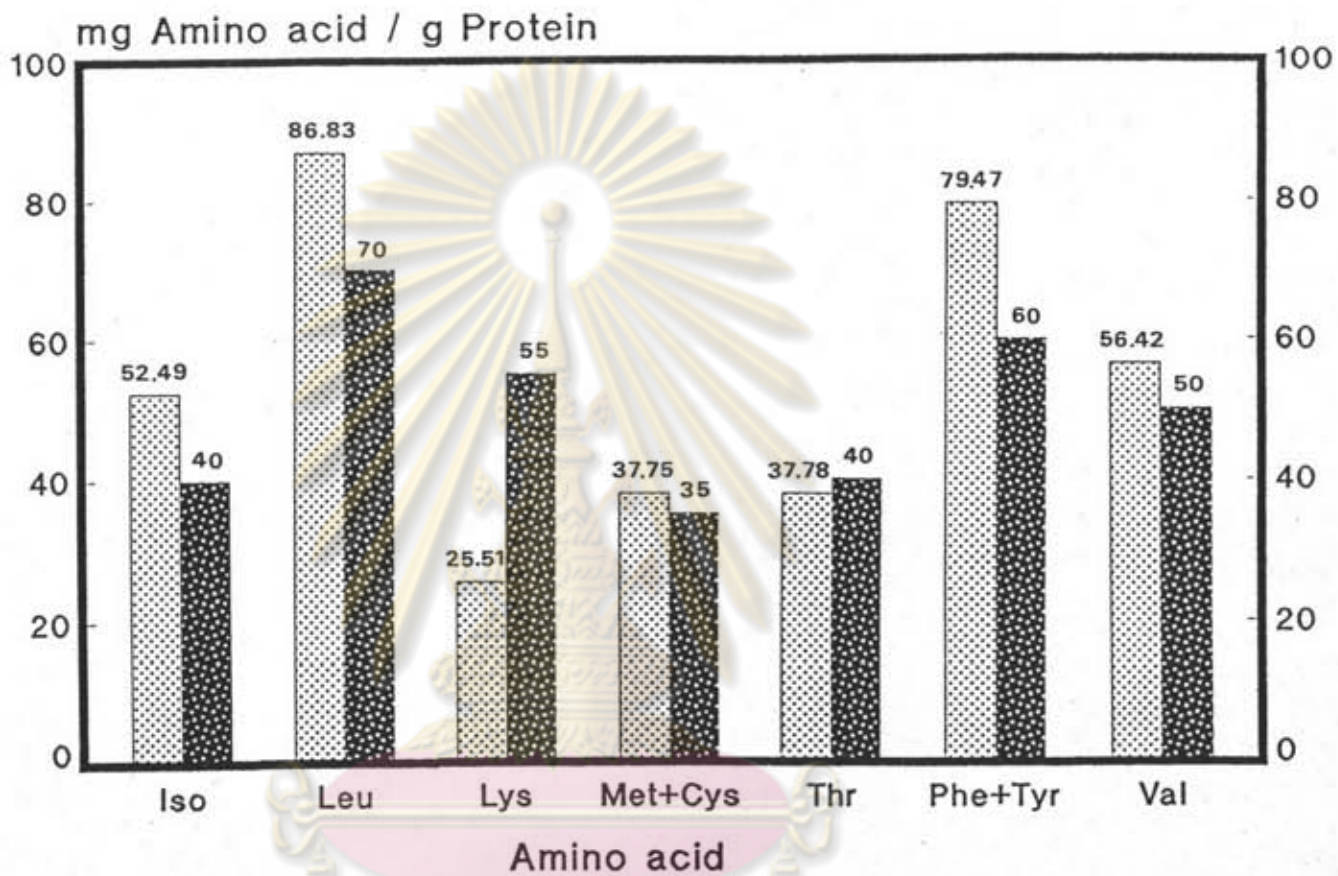
Table 6 Some Amino Acid Scores of Spray-dried Low Lactose Milk-based Medical Food

Amino Acids	Sample (mg/g protein)	FAO/WHO Standard [*] (mg/g protein)	Amino Acid Scores ^{**}
Isoleucine	52.49	40	1.31
Leucine	86.83	70	1.24
Lysine	25.51	55	0.46
Methionine + Cystine	37.75	35	1.08
Threonine	37.78	40	0.94
Phenylalanine+Tyrosine	79.47	60	1.32
Valine	56.42	50	1.13

* From Joint FAO/WHO Ad Hoc Expert Committee, Energy and Protein Requirement, WHO Tech, Rep. No 522, Geneva, Switzerland, 1973

** amino acid score = $\frac{\text{mg of amino acid per g of test protein}}{\text{mg of amino acid per g protein in reference protein}}$

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Sample
 FAO/WHO Standard

Figure 4 Comparison of essential amino acid contents in spray-dried low lactose milk-based protein with FAO/WHO standard pattern

2.83 ± 0.44 . Statistical evaluation, however, did not reveal a significant difference between two investigated group ($p > 0.05$) (Table 12).

4.2 Determination of Net Protein Ratio

In this study, a zero protein control group was used for NPR determination. The average weight loss of a zero protein control animal for NPR calculation was 3.9 ± 4.0 g. Results of NPR determined in three groups of rats are shown in Table 8. Mean NPR values of casein reference group, low lactose group and lactose group were 4.27 ± 0.53 , 4.05 ± 0.80 and 4.67 ± 0.69 , respectively. Statistical comparison showed that differences was not significant for three groups ($p > 0.05$). The NPR of low lactose group and lactose group expressed as RNPR relative to the NPR of casein reference standard group were 94.80 ± 19.74 and 109.30 ± 17.07 percent, respectively. There was no significant difference between RNPR of two investigated groups ($p > 0.05$), (Table 12). The analysis of variance is shown in Table 11 (Appendix D).

4.3 Determination of Net Protein Utilization

Table 18 showed the urinary and fecal nitrogen excretion of the zero protein control group. Mean values of urinary and fecal nitrogen were 27.04 ± 9.69 and 39.33 ± 15.68 mg, respectively. True digestibilities (TD), biological values (BV) and net protein utilization (NPU) are presented in Table 9. For low lactose group and lactose group, TD, BV and NPU were not statistically different, ($p > 0.05$), (Table 12). Mean TD, BV, NPU were 95.44 ± 2.77 , 90.49 ± 1.87 , 86.36 ± 2.96 for low lactose group and 93.98 ± 3.29 , 91.77 ± 3.56 , 86.21 ± 3.83 for lactose group, respectively (Figure 5).

Table 7 Weight Gain, Food Intake, Protein Intake and PER Values of the Experimental Rats (Four-week Assay Period)

Groups	Weight Gain (g)	Food Intake (g)	Protein Intake (g)	PER	CPER*
Casein Reference	81.4 (10.2)	315.4 (27.9)	29.96 (2.65)	2.72 (0.25)	-
Low Lactose	83.6 (15.5)	264.2 (47.0)	29.73 (5.28)	2.85 (0.48)	2.62 (0.47)
Lactose	88.9 (16.6)	281.2 (23.7)	29.36 (2.47)	3.07 (0.48)	2.83 (0.44)

values presented as means (S.D.)

* determined PER for reference standard casein = 2.72

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Table 8 Weight Gain, Food Intake, Protein Intake and NPR values of the Experimental Rats (Ten-day Assay Period)

Groups	Weight Gain (g)	Food Intake (g)	Protein Intake (g)	NPR	RNPR*
Zero Protein Control	-3.9 (4.0)	45.3 (5.6)	0	-	-
Casein Reference	30.6 (6.0)	85.2 (13.7)	8.10 (1.30)	4.27 (0.53)	- -
Low Lactose	29.5 (7.2)	74.9 (16.9)	8.43 (1.90)	4.05 (0.80)	94.80 (19.74)
Lactose	26.4 (6.5)	63.8 (17.5)	6.66 (1.83)	4.67 (0.69)	109.30 (17.07)

values presented as means (S.D.)

* NPR reference = 4.27

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Table 9 Nitrogen Intake, Urinary and Fecal Nitrogen, True Digestibilities, Biological Values and Net Protein Utilization of the Experimental Rats (Ten-day Assay Period)

Groups	Nitrogen (mg)			TD	BV	NPU
	Intake	Urine	Feces			
Zero Protein Control	0	27.04 (9.69)	39.33 (15.68)	-	-	-
Low Lactose	1348.56 (304.13)	149.34 (36.93)	103.80 (48.83)	95.44 (2.77)	90.49 (1.87)	86.36 (2.92)
Lactose	1065.33 (292.03)	106.60 (30.11)	112.21 (47.95)	93.98 (3.29)	91.77 (3.56)	86.21 (3.83)

values presented as means (S.D.)

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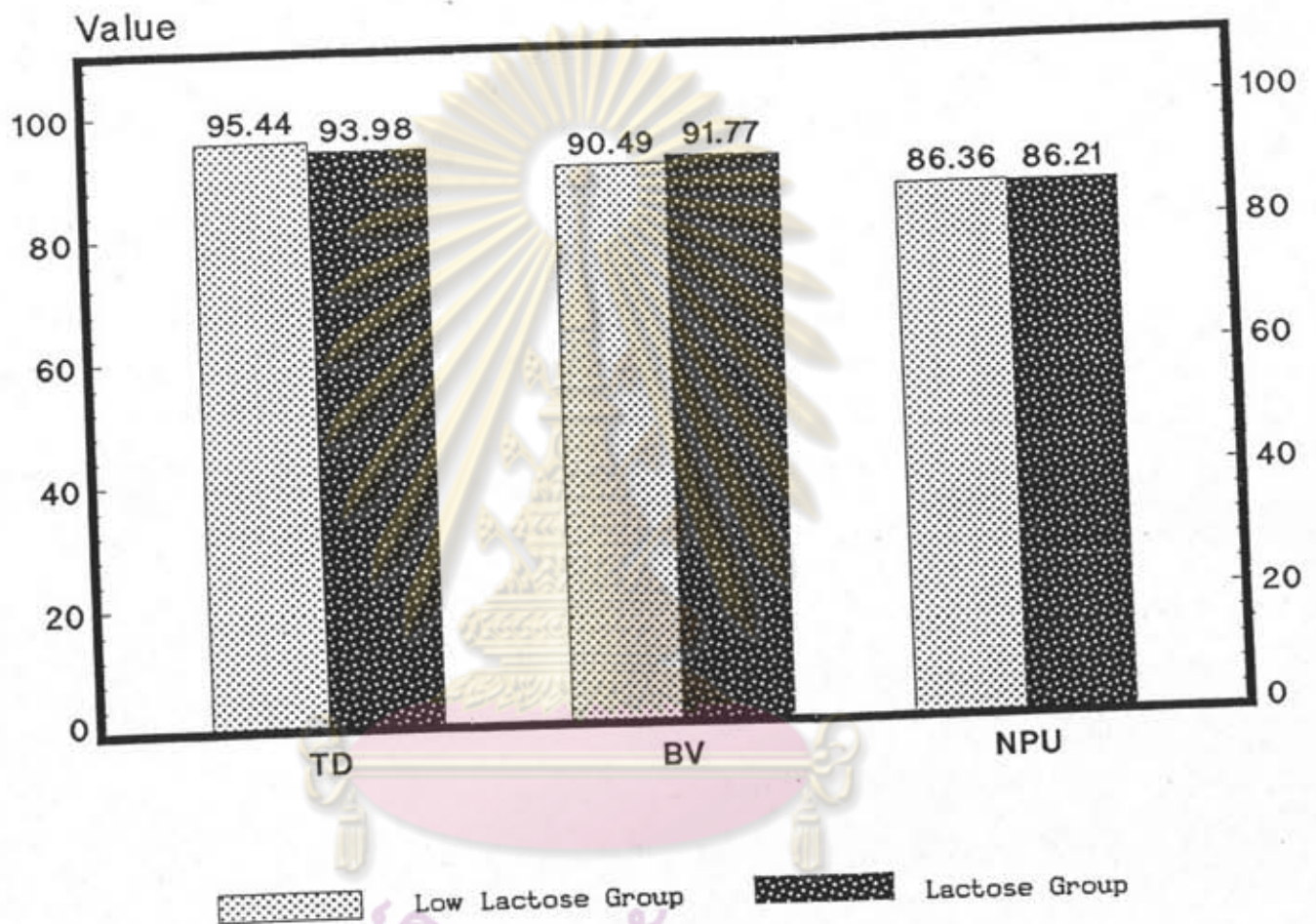


Figure 5 Comparison of mean TD, BV and NPU between low lactose group and lactose group