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PROGRESS REPORT NO.1

# DEGRADATION OF UNDESIRABLE COMPONENTS IN SOYBEAN WHEY

USAID/PSTC PROGRAM

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Submitted by

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# PROJECT PROFILE

Country : Thailand

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A. Background :

Soybean whey is a nutritional liquid waste derived from the production of soybean curd (tofu) and soy isolate (soy protein). There have been a great problem in discarding this waste all over the world because the increasing manufacturing of soybean products. An attempt to utilize soybean whey as food will greatly reduce the cost of waste treatment and impart the valuable source of protein food for the world population.

There are some objectionable components in soybean whey derived from soybean namely beany favours and flatus inducing $\alpha$-oligosaccharides. This project aims at getting rids of these undesirable components by the safe and selected microorganisms. The treated soybean whey will subsequently be utilized to produce a nutritious beverage product.

B. Objectives :

1) To Select the safe and suitable microorganisms to breakdown the indigestible -oligosaccharides : melibiose, raffinose and stachyose and modify beany flavours to the acceptable ones in soybean whey.

2) To produce an acceptable beverage from the treated soybean whey.

C. Materials and Methods :

C.1 Samples

Soybean $whey_1$ (produced in our laboratory)

Soybean $whey_2$ (taken from tofu factory)

C.2 Microoganisms

C.2.1 Microoganisms obtained from The fermented pork project. They are Gram + rods and cocci, catalase negative, presumed to be in lactic acid bacteria group. These isolates are no. 4402, 4405, 4406, 4409, 4411, 4412, 4416,4417, 4423, 4425, 4427, 4428

C.2.2 Isolates obtained from fermented natto : isolate A and isolate B

C.2.3 Isolate obtained from Biology Department Faculty of Science, CMU. being regarded as photosynthetic bacteria : isolate $P_1$

C.2.4 Leuconostoc mesenteroides B35 from TISTR, Thailand

C.2.5 Saccharomyces cerevisiae 3051 from National Food Research Institute, Tsukuba, Japan.

C.3 Media

C.3.1 Soybean $whey_1$ and soybean $whey_2$(1) prepared by precipitating protein in soymilk by $MgSo_4$ 0.2% (final concentration); the filtrate obtained after filtering was soybean whey.

C.3.2 Yeast glucose chalk litmus milk for maintenance of lactic acid bacteria.

C.3.3 MRS broth (Difco) for lactic acid bacteria cultures.

C.3.4 0.1 M $KH_2PO_4$ pH 4.7 for washing cells and preparing cell dilution.

C.3.5 Medium No.3 agar and broth for yeast culture[1].

C.4 Methods

C.4.1 Odour by smelling

C.4.2 pH measurement by pH meter PHM 61 Radiometer Copenhagen.

C.4.3 Turbidity by reading OD at 660 or 550 nm on spectronic 20

C.4.4 Reducing sugar[2]

C.4.5 Total carbohydrate[3]

C.4.6 Protein[4]

C.4.7 Total amino acids analysed by

- pipette 2 ml soybean whey sample into a test tube
- add 10% trichloro acetic acid 5-6 drops
- stand for 30 min
- centrifuge at 3000 rpm 25 min
- detect the total amino acid in supernatant by pipetting 5 ml 0.4 M $Na_2CO_3$ and 1 ml Folin - Ciocateau phenol reagent into 1 ml protein-free soybean whey prepared above. Mix

well. Incubate at 40°C in water-bath for 30 min. Read optical density (OD) at 660 nm with Bausch & Lomb spectronic 21. Use tyrosine as a standard.

C.4.8 Method of culturing different microorganisms[1]

D. Results, Discussion and Tables :

D.1 Inoculum Size.

The numbers of cells in inocula used as starter organisms presented on Table 1 and 2 were Leuconosoc mesenteroides B35 and Saccharomyces cerevisiae 3051 at 25°C being $4.2 \times 10^{10}$ cells/g (wet wt.) or $4.2 \times 10^{8}$/ml at OD = 0.678 at 550 nm (Table 1) and $1.5 \times 10^{10}$ cells/g (wet wt.) or $1.5 \times 10^{7}$/ml at OD = 0.456 at 550 nm (Table 2) respectively. The reading OD at $10^{6}$/ml of the applied microorganisms will be confirmed again next period.

D.2 Physical and Biochemical Properties of Investigated Microorganisms In Soybean Whey.

Chemical properties such as pH, acidity, reducing sugar, total carbohydrate, total amino acids and protein including physical properties such as odour of original soybean whey (non-sterile and sterile) together with the ones fermented with Leuconostoc mesenteroides B35 and Saccharomyces cerevisiae 3051 at 25°C for 9 days were shown on Table 3 and 4

Original soybean whey used in these experiments was taken from the tofu factory in Chiang Mai. They gave soybean whey odour similar to beany flavour. After they were autoclaved at 121°C 15 min, their acidity was slighty increased whereas pH, reducing sugar, total carbohydrate, total amino acids and protein were decreased (Table 3). Leuconostoc mesenteroides B35 could ferment soybean whey actively. It changed their flavour into sweet fruity odour and gave higher acidity to 0.33% with pH 4.3 in 1 day. After fermentation for 1 days pH and acidity did not change much whereas reducing sugar, total carbohydrate, total amino acid and protein kept changing throughout 9 days (Table 3).

Table 4 illustrated the activity of Saccharomyces cerevisiae3051 in soybean whey. This organisms showed soy sauce odour and increased the pH of soybean whey since the 3th days and brought up to pH 9.4 in 7 days of fermentation. After 1 day its growth was slightly changed along with assimilating reducing sugar, total carbohydrate, amino acids and protien slowly.

Table 1

Numbers of Cells of *Leuconostoc mesenteroides* B35 And Turbidity at 25°C

| Dilution | Colonies on MRS agar | | | | | | Turbidity | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | ave. | 660nm | 550nm |
| $10^{-1}$ | - | - | - | - | - | - | 1.5 | 1.523 |
| $10^{-2}$ | - | - | - | - | - | - | 0.658 | 0.678 |
| $10^{-3}$ | - | - | - | - | - | - | 0.085 | 0.086 |
| $10^{-4}$ | - | - | - | - | - | - | 0.010 | 0.013 |
| $10^{-5}$ | - | - | - | - | - | - | 0 | 0.007 |
| $10^{-6}$ | - | - | - | - | - | - | 0 | 0.004 |
| $10^{-7}$ | *** | *** | *** | *** | *** | *** | 0 | 0.003 |
| $10^{-8}$ | >400 | >400 | >400 | >400 | >400 | >400 | 0 | 0.002 |
| $10^{-9}$ | 44 | 50 | 36 | 47 | 32 | 42 | 0 | 0.001 |

*Leuconostoc Mesenteroides* B35 = $4.2 \times 10^{10}$ cells/g (wet wt.)

- = not cultured

*** = uncountable

Table 2

Numbers of Cells of *Saccharomyces cerevisiae* 3051 And Turbidity at 25°C

| Dilution | Colonies on Medium No.3 agar | | | | | | Turbidity | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | ave. | 660 nm | 550 nm |
| $10^{-1}$ | – | – | – | – | – | – | 1.5 | 1.512 |
| $10^{-2}$ | – | – | – | – | – | – | 0.08 | 0.770 |
| $10^{-3}$ | – | – | – | – | – | – | 0.425 | 0.456 |
| $10^{-4}$ | – | – | – | – | – | – | 0.018 | 0.022 |
| $10^{-5}$ | – | – | – | – | – | – | 0 | 0.005 |
| $10^{-6}$ | – | – | – | – | – | – | 0 | 0.003 |
| $10^{-7}$ | *** | *** | *** | *** | *** | *** | 0 | 0.003 |
| $10^{-8}$ | 160 | 175 | 156 | 170 | 167 | 166 | 0 | 0.002 |
| $10^{-9}$ | 14 | 19 | 15 | 13 | 16 | 15 | 0 | 0.001 |

*Saccharomyces cerevisiae* 3051 = $1.5 \times 10^{10}$ cells/g (wet. wt.)

– = not cultured

Table 3

Properties of Soybean Whey Before And After Fermented With *Leuconostoc mesenteroides* B35 at 25°C

| Properties | original soybean whey | | Soybean Whey Fermented With *Lenconostoc mesenteroides* B35 (days) | | | | |
|---|---|---|---|---|---|---|---|
| | * 1 | * 2 | 1 | 3 | 5 | 7 | 9 |
| Odour | SO | SO | FO++ | FO++ | FO++ | FO++ | FO++ |
| pH | 6.2 | 5.7 | 4.3 | 4.2 | 4.2 | 4.2 | 4.2 |
| %Acidity | 0.029 | 0.055 | 0.331 | 0.359 | 0.381 | 0.381 | 0.381 |
| %Reducing Sugar | 0.118 | 0.049 | 0.099 | 0.109 | 0.114 | 0.120 | 0.125 |
| %Total Carbohydrate | 0.77 | 0.55 | 0.395 | 0.345 | 0.297 | 0.212 | 0.172 |
| %Total Amino Acids | 0.028 | 0.025 | 0.022 | 0.020 | 0.017 | 0.015 | 0.012 |
| %Protein | 0.478 | 0.30 | 0.240 | 0.212 | 0.192 | 0.162 | 0.100 |

* 1 = non-sterile original soybean whey
* 2 = sterile original soybean whey
SO = soybean whey odour
FO = fruity odour

Table 4

Properties of Soybean Whey Before And After Fermented With *Saccharomyces cerevisiae* 3051 AT 25°c

| Properties | Sterile Original Soybean Whey | Soybean Whey Fermented With *Saccharomyces cerevisiae* 3051 (day) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 5 | 6 | 7 | 9 |
| Odour | SO | SSO+ | SSO++ | SSO++ | SSO++ | SSO+++ | SSO+++ |
| pH | 6.4 | 6.2 | 8.4 | 8.5 | 8.5 | 9.4 | 9.4 |
| %Acidity | 0.0244 | 0.031 | - | - | - | - | - |
| Turbidity(660nm) | 0.137 | 1.25 | 1.45 | 1.50 | 1.50 | 1.52 | 1.52 |
| %Reducing Sugar | 0.149 | 0.138 | 0.082 | 0.062 | 0.060 | 0.056 | 0.054 |
| %Total Carbohydrate | 0.600 | 0.320 | 0.288 | 0.260 | 0.212 | 0.188 | 0.170 |
| %Total Amino Acids | 0.027 | 0.025 | 0.024 | 0.023 | 0.022 | 0.022 | 0.021 |
| %Protein | 0.380 | 0.360 | 0.345 | 0.340 | 0.332 | 0.320 | 0.315 |

SO = Soybean Odour

SSO = Soy Sauce Odour : +, little; ++, strong; +++, stronger.

Table 5

Biochemical Changes of Soybean Whey After Fermented With Some Microorganisms At 32°C For 2 Days

| Organisms | Soybean Whey$_1$ | | | Soybean Whey$_2$ | | |
|---|---|---|---|---|---|---|
| | pH | %Total Carbohydrate | %Reducing Sugar | pH | %Total Carbohydrate | %Reducing Sugar |
| Control | 5.82 | 0.710 | 0.132 | 6.17 | 0.900 | 0.081 |
| 4405 | 3.52 | 0.358 | 0.020 | 3.40 | 0.382 | 0.020 |
| 4409 | 5.20 | 0.615 | 0.068 | 5.00 | 0.675 | 0.122 |
| 4411 | 4.62 | 0.550 | 0.095 | 5.28 | 0.580 | 0.079 |
| 4412 | 3.26 | 0.480 | 0.105 | 3.26 | 0.290 | 0.113 |
| 4423 | 4.50 | 0.532 | 0.157 | 3.50 | 0.320 | 0.187 |
| 4425 | 5.12 | 0.590 | 0.120 | 5.25 | 0.520 | 0.093 |
| 4427 | 3.38 | 0.330 | 0.143 | 3.35 | 0.330 | 0.156 |
| 4402 | 3.62 | 0.018 | 0.012 | 3.82 | 0.068 | 0.006 |
| 4406 | 3.60 | 0.088 | 0.006 | 3.80 | 0.040 | 0.008 |
| 4416 | 4.22 | 0.545 | 0.010 | 4.10 | 0.552 | 0.006 |
| 4417 | 3.92 | 0.215 | 0.011 | 3.95 | 0.312 | 0.010 |
| 4428 | 3.55 | 0.370 | 0.006 | 3.60 | 0.425 | 0.006 |
| A | 4.40 | 0.052 | 0.004 | 4.52 | 0.040 | 0.004 |
| B | 4.82 | 0.025 | 0.002 | 5.15 | 0.025 | 0.003 |
| $P_1$ | 5.60 | 0.678 | 0.004 | 5.98 | 0.675 | 0.006 |

Soybean Whey$_1$ = Soybean whey prepared in our laboratory.

Soybean Whey$_2$ = Soybean whey prepared by the [illegible]

Table 6

Biochemical Changes of Soybean Whey After Fermented With Some Microorganisms At 37°C For 2 Days

| Organisms | Soybean Whey$_1$ | | | Soybean Whey$_2$ | | |
|---|---|---|---|---|---|---|
| | pH | %Total Carbohydrate | %Reducing Sugar | pH | %Total Carbohydrate | %Reducing Sugar |
| Control | 5.82 | 0.710 | 0.132 | 6.17 | 0.900 | 0.081 |
| 4405 | 3.68 | 0.412 | 0.021 | 3.45 | 0.370 | 0.018 |
| 4409 | 3.75 | 0.438 | 0.198 | 3.68 | 0.350 | 0.184 |
| 4411 | 4.50 | 0.465 | 0.177 | 4.30 | 0.595 | 0.047 |
| 4412 | 3.32 | 0.322 | 0.112 | 3.32 | 0.300 | 0.121 |
| 4423 | 3.35 | 0.350 | 0.178 | 4.20 | 0.388 | 0.153 |
| 4425 | 4.85 | 0.472 | 0.120 | 4.68 | 0.532 | 0.085 |
| 4427 | 3.36 | 0.320 | 0.162 | 3.48 | 0.365 | 0.157 |
| 4402 | 3.45 | 0.025 | 0.016 | 3.45 | 0.112 | 0.005 |
| 4406 | 3.55 | 0.115 | 0.013 | 3.50 | 0.100 | 0.003 |
| 4416 | 3.90 | 0.458 | 0.014 | 3.95 | 0.438 | 0.006 |
| 4417 | 3.52 | 0.320 | 0.012 | 3.68 | 0.558 | 0.004 |
| 4428 | 3.58 | 0.202 | 0.008 | 3.68 | 0.380 | 0.004 |
| A | 4.70 | 0.018 | 0.006 | 4.08 | 0.065 | 0.006 |
| B | 4.55 | 0.040 | 0.009 | 3.90 | 0.135 | 0.010 |
| $P_1$ | 5.60 | 0.575 | 0.007 | 6.00 | 0.725 | 0.008 |

Soybean Whey$_1$ = Soybean whey prepared in our laboratory.

Soybean Whey$_2$ = Soybean whey prepared by the tofu factory.

Table 5 compared the biochemical changes of soybean $whey_1$ and soybean $whey_2$ when fermented by 15 isolates at 32°C. There were little difference in pH, total carbohydrate and reducing sugar in soybean $whey_1$ and soybean $whey_2$. Both soybean whey samples showed the same characteristics after all fermentation by different isolates. Most interested isolates were 4402, 4406, 4428, A and B because They could reduce the amount of total carbohydrate significantly and produced sufficient acidity (giving pH below 4.5). Isolate 4405, 4412, 4423, 4427, 4416, 4417 gave sufficent acid but reduced the amount of total carbohydrate less than the former 5 isolates. Isolate 4409, 4411, 4425 could not produce enough acid. The photosynthetic bacteria ($P_1$) hardly produced acid and digest total carbohydrate together with reducing sugar. These organism might not like the condition in soybean whey. Some microorganisms in these experiments could increase the amount of reducing sugar potentially derived from breaking down some oligosaccharides in soybean whey.

Table 6 illustrated the same experiments as Table 5 but fermentation was carried out at 37°C. The results in this experiment confirmed the test at 32°C (Table 5). Most unknown lactic acid bacteria applied produced higher acidity (giving lower pH) than at 32°C and isolate 4409, 4411, 4425 imparted sufficient acidity at 37°C whereas photosynthetic bacteria ($P_1$) showed the same result as at 32°C

## E. Conclusion

Most experiments in this period are preliminary studied. The results however supported the tendency of the achievement of the project objective : there will be some microorganisms able to utilize the indigestible $\alpha$-oligosaccharides and modify the beany odour of soybean whey.

## F. Workplan for the Next Period :

1 Standardize the inoculum size.

2 Investigate the fermentation of melibiose in carbohydrate-free MRS broth by more lactic acid bacteria at different temperatures.

3 Investigate the melibiose fermentation by some more aerobic microorganisms in appropriate fermentation medium.

4 Quantify the amount of stachyose, raffinose and melibiose in original soybean whey and after fermentation by paper chromatography.

5 Characterize the odour of soybean whey after different fermentation by at least 2 trained panels.

6 Determine the proximate analyses of soybean whey.

G. References :

1. Pinthong R., H-Kittikun, A., Klunklin G., and Silprasert A. (1986) Degradation of Undesirable Components in Soybean Whey. AID/ACI PSTC Research Proposal. Project Identification No, 7.303 Chiang Mai University, Chiang Mai, Thailand P.9,27, 29 and 31.

2. Miller, G.L. (1956). Use of Dinitrosalicylic Acid Reagent for the Determination of Reducing Sugar. Anal. Chem. 31 : 426-428.

3. Dubois, M.; Gillies, K.A.; Hamilton, J.K.; Rebers, P.A.; and Smith, F. (1956). Colorimetric Method for Determination of Sugar and Related Substances. Anal. Chem. 28 : 350-356.

4. Lowry, O.H.; Rosenbrough, J.J.; Farr, A.L.; and Randall, R.J. (1951). Protein Measurement with the Folin-phenol Reagent. J. Biol. Chem. 193 : 256-275.

H. Pictures :

Plate 1 Dr. Renu Pinthong (right).

Dr. Arun H-Kittikun (center), Dr. Karun Klunklin (left) with a gas liquid chromatograph at Dept. of Chemistry, CMU.

Plate 2 Mr. Adung Silprasert with amino acid analyser at Institute of Health Science, CMU.

Plate 3 Mr. Adung Silprasert (center); Miss Nonglung Chompoo, technichian (left); Miss Chulaluk Tepboon, technician (right). At the Institute of Health Science, CMU.

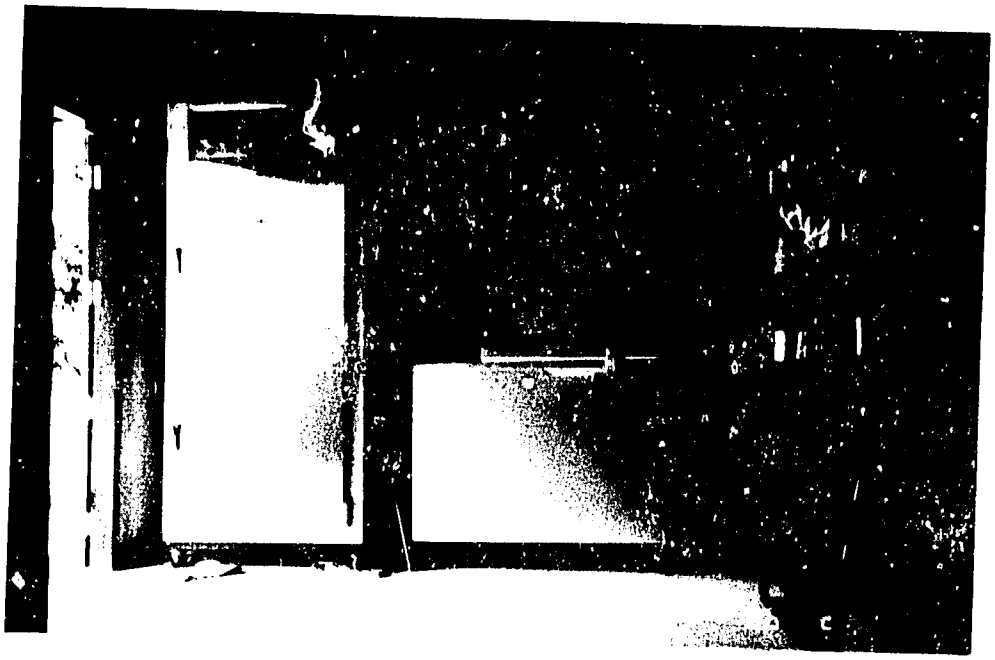

Plate 4 Low temperature incubator (0-25°C) (left) and freezer 0°C to 0-20°C (right) at Dept. of Food Science and Technology, CMU.

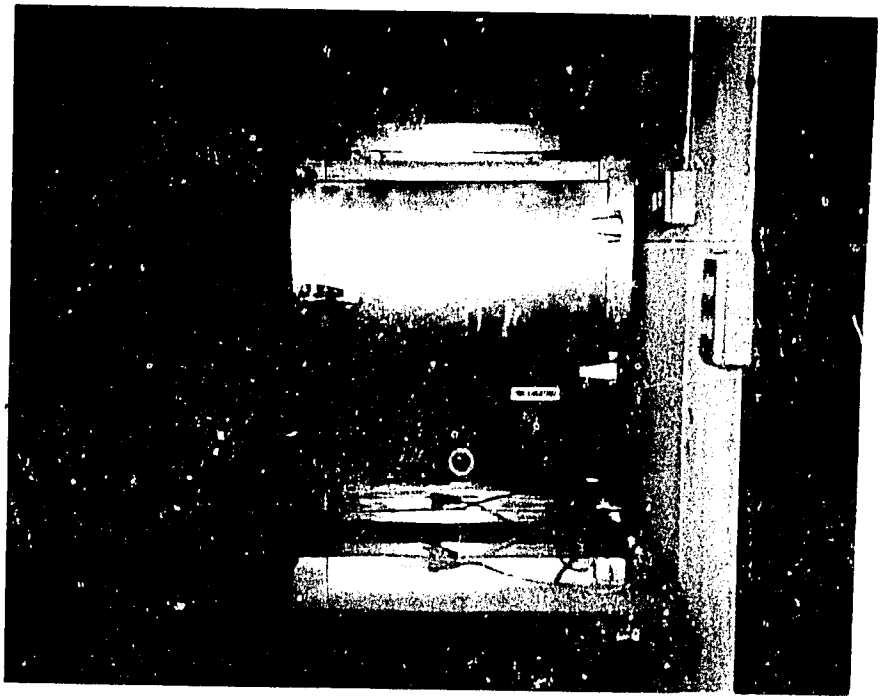

Plate 5 High temperature incubator (room temperature to 60°C) at Dept. of Food Science and Technology, CMU.

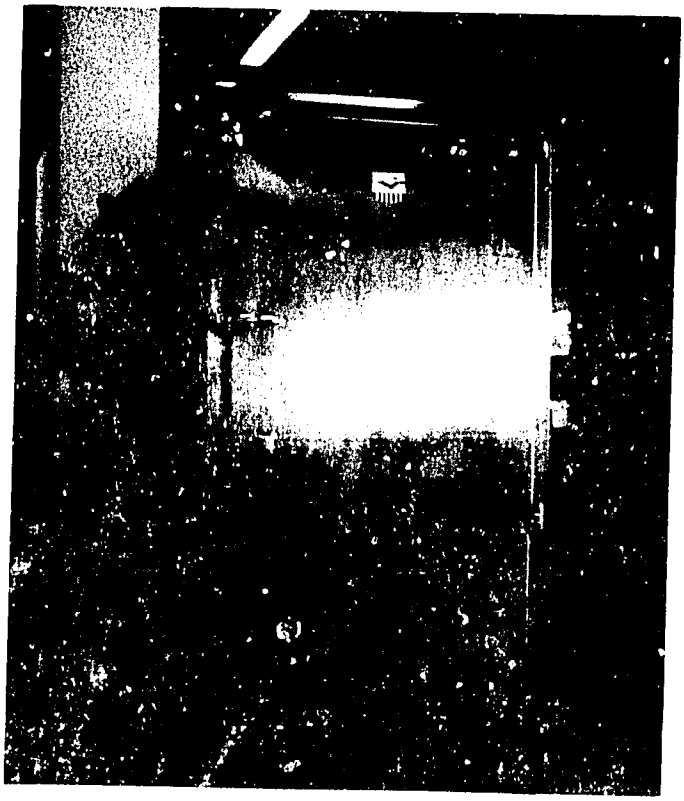

Plate 6 Drying oven (room temperature-150°C) at Dept. of Food Science and Technology, CMU.

Plate 7 Tofu Factory belong to Mr. Artorn at Mae Joe, Chiang Mai

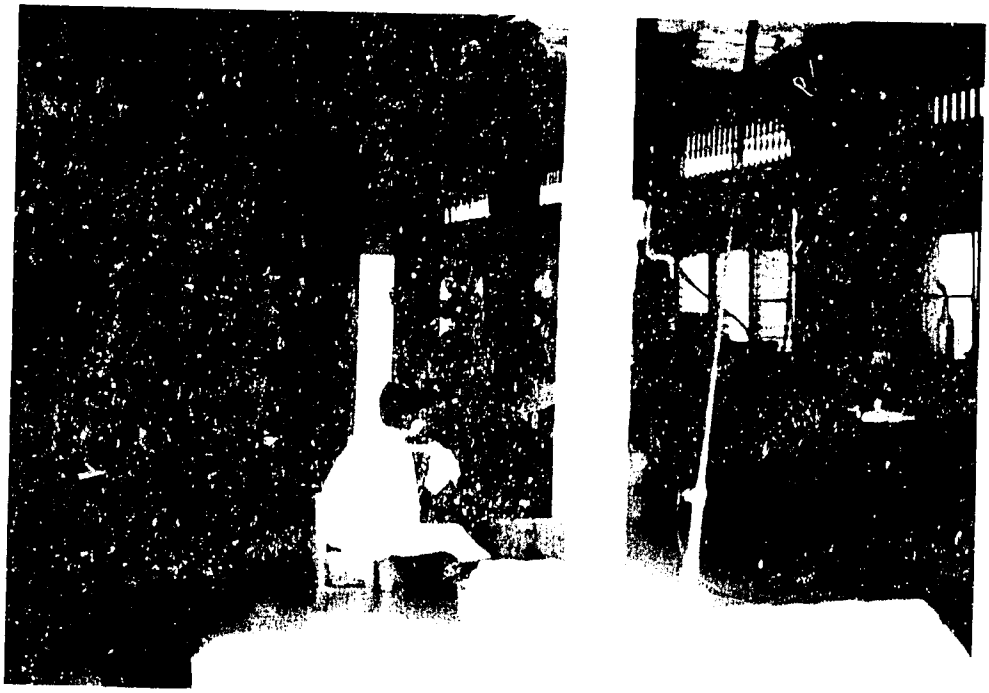

Plate 8 Working condition inside the Tofu Factory.

Plate 9 Precipitation of protein from soymilk inside the tofu factory.

Plate 10 Placing the curd in tofu mould inside the tofu factory.