APPENDIX A

Picture of Imitated Milk System ingredients

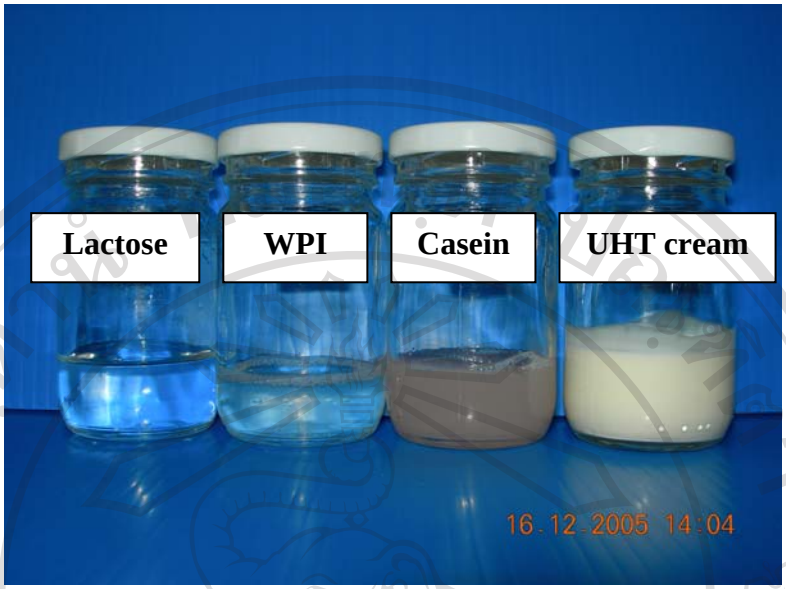

Figure A-1 Ingredients of IMS solutions

Figure A-2 UHT milk and imitate milk systems solutions

APPENDIX B

Method for chemical analysis

1. Total titrable acidity analysis (Marshall, 1992)

Measured 9 g sample into a 100 ml beaker. Added 0.5 ml of phenolphthalein indicator and titrated with 0.1 N NaOH until the first permanent color change to pink for 30 s.

Calculated the amount of lactic acid in the milk samples as fallowed:

$$\% \text{ lactic acid} = \frac{\text{ml of 0.1N NaOH} \times 0.1 \times 9}{\text{weight of sample}}$$

2. pH measurement (Marshall, 1992)

For the pH measurement, a pH meter (Consort C830, CE, Belgium) was turned on one and half hour before used. The pH meter was standardized with buffer solutions including pH 4.0 and 7.0 before determination was made. Immersed the electrode directly into the sample and made sure the electrode contacted with the sample until the reading was stabilized. Read the pH directly.

APPENDIX C

Method for microbiological analysis

1. Total Viable Microorganisms count (Marshall, 1992)

Shaked samples and wiped the top of unopened samples container with a sterile cotton saturated with 70% ethyl alcohol. After made dilution using 9 ml Maximum Recovery Dilution (MRD) (Oxoid, England) and plated on Plate Count Agar (PCA) (Merck, Germany), the plates were incubated at 30°C for 2 days.

2. Spore count (Harrigan, 1998)

Pipetted 5-10 ml of well mixed sample into a sterile test tube. Heated the sample in a water bath at 80ºC for 10 min using a control tube that contained a thermocouple probe inside it to monitor the temperature changing in the milk sample . The 10 min heating time was started after the thermocouple probe in the control tube showed a temperature of 80°C. Cooled the sample and plated on Plate Count Agar (PCA) (Merck, Germany). The plates were incubated at 30°C for 2 days for mesophiles spore.

3. Thermoduric bacteria count (Harrigan, 1998)

Mixed the sample and placed 10 ml of the sample into a sterile test tube. Immersed the tube of samples in a water bath at 63.5±0.5°C for 30 min. After the time finished, the samples were removed from the water bath and cooled rapidly in ice water. The milk samples were diluted as appropriately and plated on Plate Count Agar (PCA) (Merck, Germany). The plates were incubated at 30°C for 2 days.

APPENDIX D

Method for nisin assay

Nisin assay

Bioassay preparation ((Pongtharangkul and Demirci, (2004) with some modification).

A nisin sentitive microorganisms used in this study was *Micrococcus luteus*, which was obtained from Thailand Institute of Scientific and Technological Research. The stock culture was maintained at 4ºC and grown in nutrient broth (NB; Oxoid, England) for 24 h at 30ºC. The bioassay agar plates used to measure nisin activity was made from NA and 1% Tween 20. The agar was heated until it was melted and sterilized at 121ºC for 15 min. After the sterilization process, the agar medium was cooled to 40ºC and inoculated with 1% of *M. luteus* suspension that had been grown for 24 h at 30ºC in NB. The young culture of *M. luteus* contained 5 log cfu/ml in NB. The microbial number was checked regularly to ensure that the inoculated culture in each experimental section had a microbial number of at least 10^5 cfu/ml in the NA medium. An amount of 25 ml of the bioassay agar was poured into sterile petri dishes and allowed to solidity for 3 h. For each of the plates, four or five holes were bored using a 7 mm outer diameter stainless steel borer. Three different replication for each treatment sample were evaluated in each of the experimental section. An aliquot 100 µl of each standard nisin solution or milk sample was placed into a well and the plates were incubated at 30ºC for 24 h. The diameter of the inhibition zone around each well was measured.

Standard nisin solution preparation

Weighted 0.1 g of nisin (Nisapin) and then transferred to a 100 ml volumetric flask, rinsed and made up the volume to approximately 60 ml used 0.02 N HCl. Placed the flask in a boil water bath cover the neck with a piece of aluminum foil and weight down the flask with a lead ring and heated for 10 min. After heated, removed the flask and cooled to the room temperature by immersed in cold water. Made up the cooled solution to 100 ml with more 0.02 N HCl. The solution should be cooled to 5ºC in a refrigerator and had to be left for a minimum of 1 h before used. This acidic suspension of nisin contained 1,000 IU/ml nisin, was quite stable and could be used

for up to 1 week if kept in a refrigerator. Mixed thoroughly before each used and allowed this liquid to come to room temperature before used. Standard nisin solutions of 150, 100, 75, 50, 25 and 0 IU/ml were prepared using 1,000 IU/ml nisin stock solution and diluent solution and used to construct the standard curve.

Prepared sample for nisin assay

An amount of samples 10 ml of the milk samples was added to 90 ml of 0.02 N HCl in a 250 ml Duran bottle. This was be mixed thoroughly by swirling. Then, the sample suspension was heated with the same condition as the standard nisin solution to determined the inhibition zone of nisin residual.

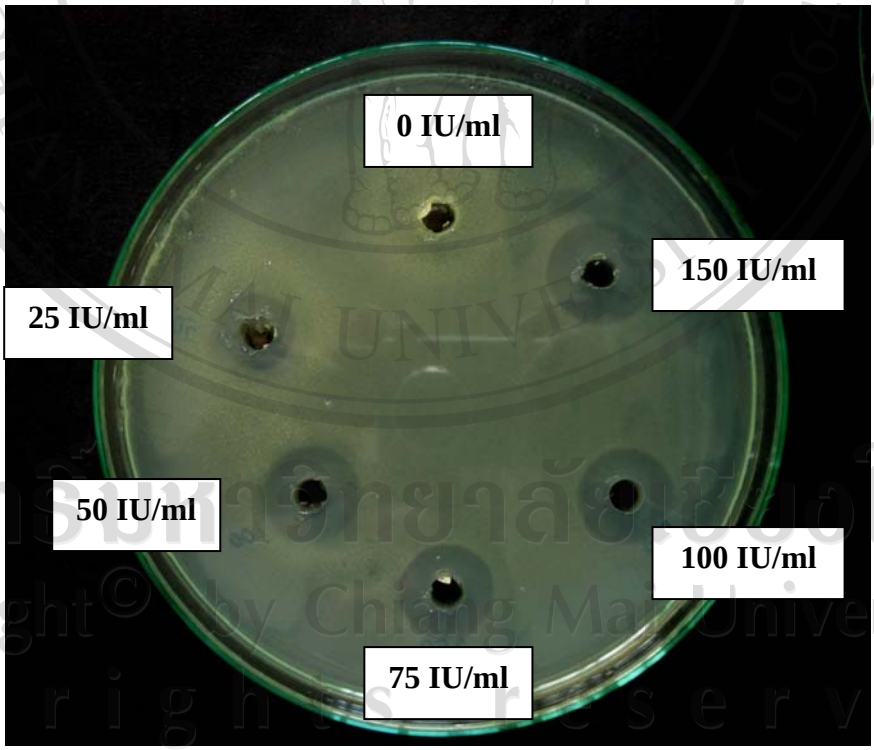

Figure D-1 Bioassay agar plate of *M. luteus* with standard nisin solutions.

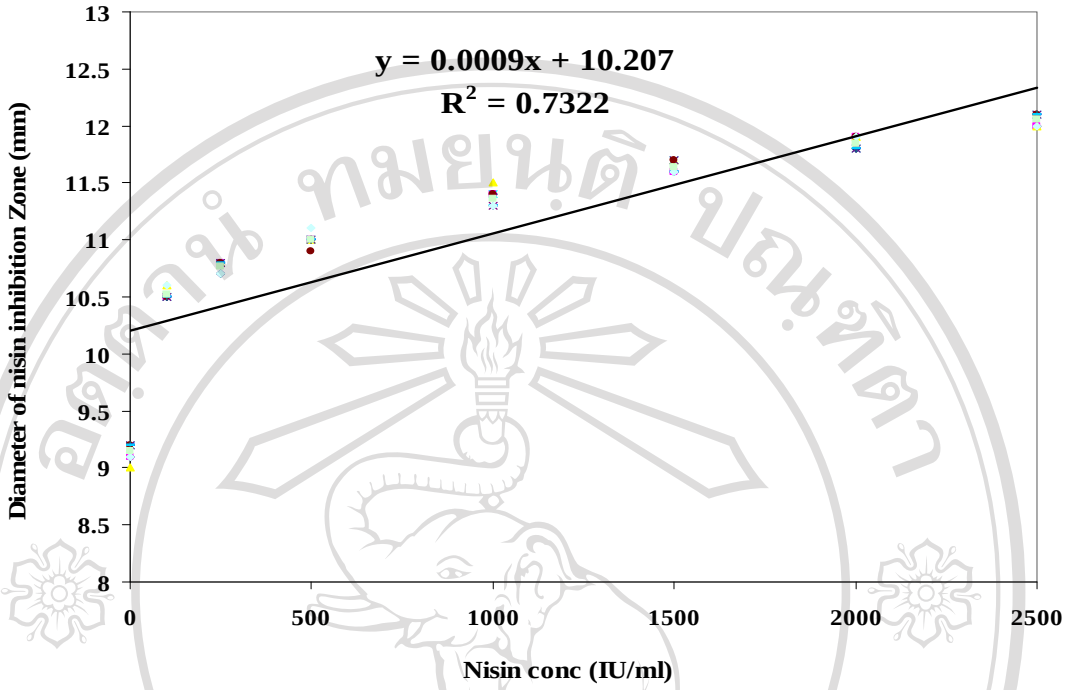

Figure D-2 Diameter of nisin inhibition zone using nisin concentrations of 0 to 2,500 IU/ml against *Micrococcus luteus* on bioassay agar plates.

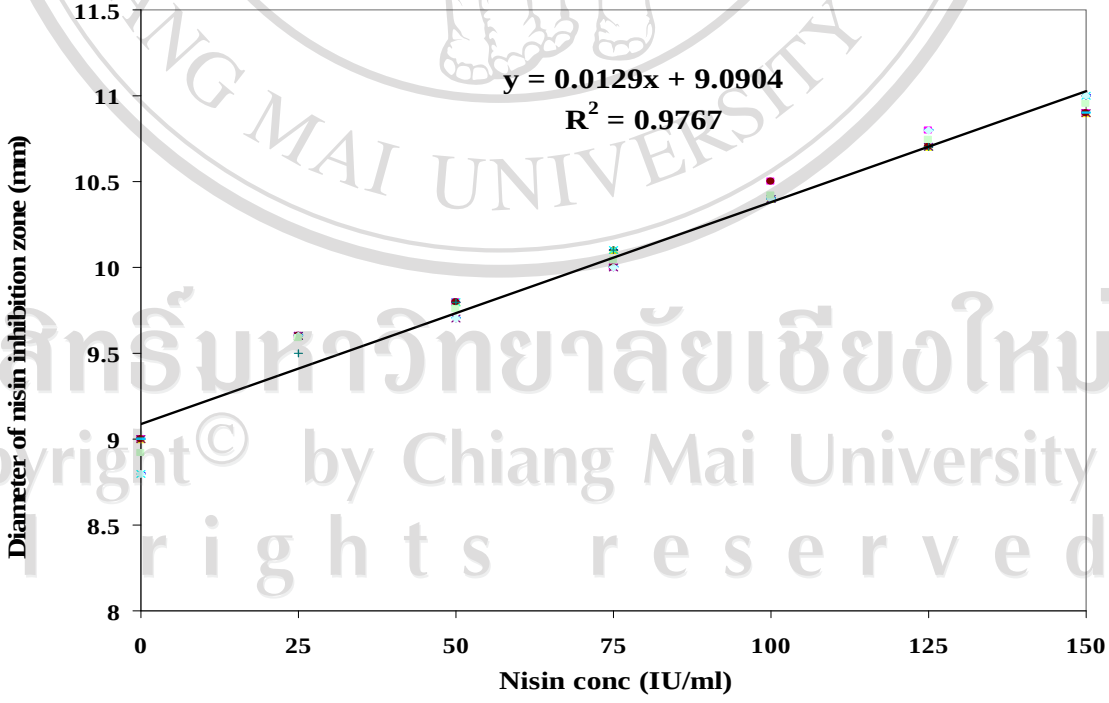

Figure D-3 Diameter of nisin inhibition zone using nisin concentrations of 0 to 250 IU/ml against *Micrococcus luteus* on bioassay agar plates

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