

Appendix A

Pictures



Figure A.1 Corn milk



A

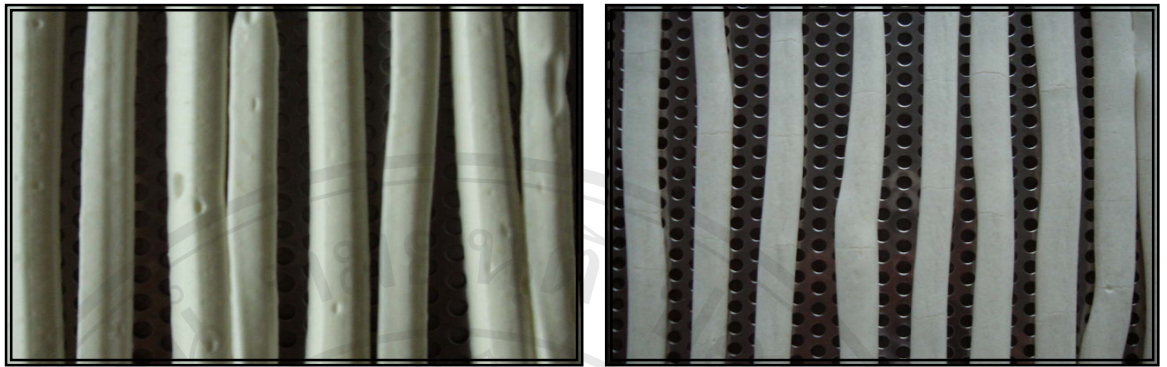


B

Figure A. 2

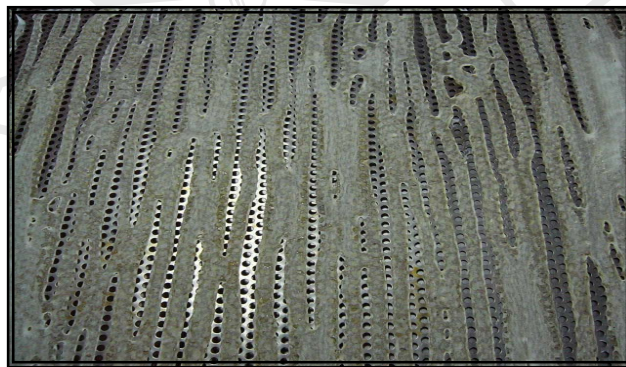
A : Stable foam

B: Unstable foam



A

B



C

Figure A. 3

A : Foam before drying

B : Stable foam after drying

C : Foam collapsed during drying



Figure A. 4 Corn milk powder produced from stable foam

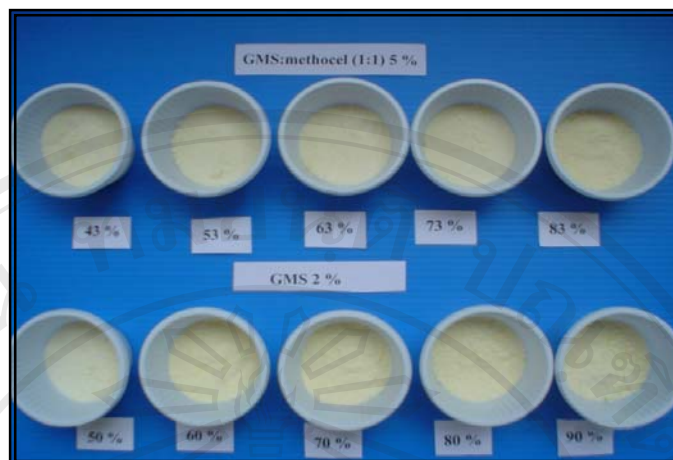


Figure A. 5 Corn milk powders prepared by 2 % concentration of GMS and 5 % concentration of GMS and methocel (1:1)

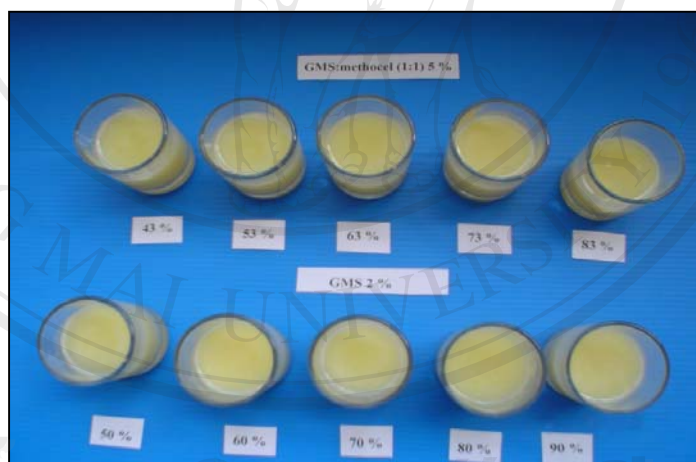


Figure A. 6 Reconstituted corn milks prepared by 2 % concentration of GMS and 5 % concentration of GMS and methocel (1:1)

Appendix B

Physical quality analysis

1. Color (Hunter lab)

Color evaluation was performed on all dehydrated and reconstituted samples using a colorimeter, Minolta Data Processor DP-301 Chroma meter. The instrument was calibrated with a white tile. A white standard tile with reflectance values of $x = 82.51$, $y = 84.53$, and $z = 101.23$ was used as a reference. The Hunter L^* , a^* , and b^* scale give a measurement of color in units of approximate visual uniformity throughout a solid. L^* value represented the lightness of color, a^* value represented the greenness or redness and b^* value represented the blueness and yellowness.

- L^* value measures lightness and varies from 100 for perfect white to zero for black.
- a^* value measures redness when positive (+) and greenness when negative (-) with maximum values of 60.
- b^* value measures yellowness when positive (+) and blueness when negative (-) with maximum values of 60.

Each value represented a mean value of three determinations for each sample.

2. Rehydration (Ratchaniyom, 2002)

Two g of corn milk powders was dissolved in distilled water at room temperature. The solution was stirred for 1 min and then it was filtered through a filter paper no. 4. Afterwards, the filter paper was dried in an oven at 100°C for 3 h to determine the insoluble solid. The percentage of rehydration was calculated according to the following equation;

$$\text{rehydration (\%)} = \frac{\text{lossed weight of corn milk}}{\text{initial weight of corn milk}} \times 100$$

3. Solubility (Sommanut, 1997)

Twenty g of corn milk powders was dissolved with 250 ml distilled water in a 500 ml beaker at room temperature. The solution was then stirred by a magnetic

stirrer at a speed level of 5. The time that was needed to completely dissolve the corn milk powders determined the solubility time and it was expressed in min.

4. Dispersibility (Sommanut, 1997)

Two g of corn milk powders was placed in a 150 ml beaker and added with 100 ml distilled water. At room temperature, the mixture was stirred using a magnetic stirrer at a speed level no 5 for 30 s. After that, 50 ml of the mixed solution was removed by a syringe and transferred into a centrifuge tube. The tube would then be centrifuged at 1740 rpm for 3 min. The supernatant or the upper part of the solution was collected and measured for its absorbent by a spectrophotometer at a wavelength of 520 nm.

5. Foam syneresis or drainage method (Sauter and Monture, 1972)

The foam was fully filled into a conical- shaped glass which was supported by a funnel and placed on a 250 ml graduated cylinder. The liquid milk which was separated from the foam due to syneresis was collected in the measuring cylinder. The amount of liquid milk collected after leaving for 3 h was recorded.

6. Foam density (Ratchaniyom, 2002)

A known volume of foam was transferred into a plate that had been measured for its own weight and weighted. The foam density was obtained by dividing the weight of the foam with the volume of the foam, which could be seen from the following equation;

$$\text{Foam density} = \frac{\text{Foam mass (g)}}{\text{Foam volume (ml)}}$$

7. Overrun (Jitjaroen, 1999)

Corn milk's mixture and foam were transferred into a plate that had been measured for its own weight and weighted. The percentage of an overrun was calculated from

$$\text{Overrun (\%)} = \frac{\text{weight of the mixture} - \text{weight of the foam} \times 100}{\text{weight of the foam}}$$

8. Hygroscopic (Sommanut, 1997)

Ten g of corn milk powders was placed on an open plate at room temperature for 7 days. Everyday, the weight of the powder was measured to calculate the rate of hygroscopic.

9. Viscosity

Ten g of corn milk was poured into a special cylinder that was used to measure viscosity. After that, place the cylinder in a viscometer, put in a viscometer stirrer and turn on the equipment. Figure out the viscosity of the corn milk samples by using a correct speed velocity for the stirrer.

Appendix C

Chemical quality analysis

1. Fat in corm milk by the Rose-Gottlieb method (a modified method from an AOCA official method no.905.02, 2000)

Apparatus

1. Separatory funnels.
2. Flasks

Reagents

1. Ammonium hydroxide (NH₄OH) (J.T. Baker, USA)
2. Petroleum ether (J.T. Baker, USA)
3. Diethyl ether (Merck, Germany)
4. Ethyl alcohol 95 % (Merck, Germany)

Determination

Weight 3 g corm milk into a separatory funnel. Add 1 ml NH₄OH and mix thoroughly. Add with 10 ml ethyl alcohol 95 % and mix well. Next, add with 25 ml diethyl ether that must be peroxide-free, and then close with a stopper, and shake very vigorously for 1 min. After that, add with 25 ml petroleum ether which has a boiling point range of 40-60 °C and repeat vigorous shaking for 30 s. Let the funnel stand until the liquid is separated into two layers. Upper liquid is separated into a flask that is dried and known accurately its weight. Repeat the extraction of the remaining liquid in the separatory funnel twice, using 15 ml diethyl ether and 15 ml petroleum ether. The upper liquid that is extracted for 3 times is added together into the dried flask. Then, the flask is taken into a hot air oven at 100 °C, and dried to a constant weight.

2. Protein in corn milk by the Kjeldahl method (a modified method from an AOAC official method no.991.20, 2000)

Apparatus

1. A macro-Kjeldahl Distillation

Reagents

1. A catalyst mixture include of 96% sodium sulfate (Fisher, UK), 3.5% Copper sulfate (Carlo Erba, Germany) and 0.5% selenium dioxide (Merck, Germany)
2. 2 % boric acid solution (Merck, Germany)
3. Methyl red (Panreac, EU) /bromocresol green (Riedel-de, Germany) indicator solution composed of methyl red 0.016 % and bromocresol green 0.083 % in ethyl alcohol.
4. 50 % sodium hydroxide solution (Merck, Germany)
5. 95-98 % sulfuric acid Nitrogen free (Merck, Germany)
6. 0.05 M sulfuric acid solution (Merck, Germany)

Determination

Weight 3 g of corn milk into a Kjeldahl digestion flask. Eight g of catalyst mixture and 20 ml sulfuric acid are added into the digestion flask. Place the flask in an incline position in a digestion machine. Start the digestion on heating low enough so that the corn milk does not foam up to the neck of the digestion flask. Next, increase the burner setting and boil until the corn milk is looked clear and then cool it to room temperature. Distilled water was added into the cooled flask. Then, transfer the corn milk that has been digested into a distilling flask. Distilled water of 400 ml is added into the distilling flask and swirl to mix. Three or four boiling chips are added into the flask too. And a methyl red/bromocresol green indicator is also added. Connect up the distillation with a delivery tube dipping below a boric solution. Make a diluted digest alkaline with 50 % sodium hydroxide solution in amount of 75 ml. And then close the tap and distille the ammonia into the boric acid solution. After about 30 ml the distillation is over, open the tap and wash down a condenser and the delivery tube into the receiver. Titrate the distillate with 0.05 M sulphuric acid.

Calculate the percentage of nitrogen in the sample (1 ml 0.05 M sulphuric acid equal to 0.0014 g nitrogen). The crude protein figure can be calculated using an appropriate factor of 6.25.

3. Ash (AOAC official Method no. 945.46, 2000)

Weight 8 g of corn milk and place on a ceramic dish that is known accurately for its weight. Heat the dish on a steam bath. Afterwards, transfer the dish into a hot air oven at 500 °C until the milk become an ash. Cool the dish in an active desiccator and weight it again. Calculate the percentage of ash in the corn milk.

4. Total acidity of corn milk by a titrimetric method (an AOAC official method no 947.05, 2000)

weight 10 g of corn milk into a 250 ml flask and dilute the milk with distilled water for 2 times of its weight. Add 2 ml phenolphthalein indicator and titrate against 0.1 M NaOH until the first persistent pink appears. Calculate the total acidity by following an equation below;

$$\text{lactic acid (\%)} = \frac{(\text{amount of NaOH (ml)} \times 100)}{\text{Amount of corn milk}} \times 0.0090$$

5. Reducing sugar by the Lane and Eynon method (Rutjanakaikan, 2001)**Determination****Apparatus**

1. a 50 ml burette that use to figure out the amount of sugar

Reagents.

1. Fehling's solution no 1. Dissolve copper sulfate ($\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$) (Carlo Erba, Germany) 69.278 g in distilled water and then adjust to 1000 ml with distilled water in a volumetric flask.
2. Fehling's solution no.2. Dissolve 100 g sodium hydroxide (Merck, Germany) and 346 g sodium potassium tartate (Univar, Australia) in distilled water and then adjust to 1000 ml with distilled water in a volumetric flask.
3. Carrez I solution. Dissolve 21.9 g zinc acetate dihydrate (Univar, Australia) in distilled water that has 3 ml glacial acetic acid (Merck, Germany) and adjust the volume to 100 ml by distilled water.

4. Carrez II solution. Dissolve 10.6 g potassium ferrocyanide (Carlo Erba, Germany) in distilled water and adjust the volume to 100 ml by distilled water.
5. HCl (Merck, Germany)
6. NaOH (Merck, Germany)

1. Preparation of corn milk.

Weight 140 g corn milk and transfer into a 250 ml volumetric flask. Add 50 ml distilled water into the flask, followed by 5 ml Carrez I solution and 5 ml Carrez II solution. Adjust the volume of the volumetric flask to 250 ml by distilled water and leave the solution for 20 min. After that, filter the solution with a Whatman filter paper no 4.

2. Analysis reducing sugar before inversion

2.1 Preliminary titration

Take the filtered solution from the preparation no 1 and transfer into a 50 ml burette, ready to be used for sugar determination. Pipette 10 ml of mixed Fehling reagents (each Fehling reagent is 5 ml) into a 250 ml flask and add glass beads (8-10 pieces). Boil the Fehling solution. When the solution has already boiled, titrate the Fehling solution with the filtered solution from the burette until the blue color of the Fehling solution almost disappears, and quickly add the Fehling solution with 1-2 drops of methylene blue indicator. After the addition, continue the titration until the blue color absolutely disappears. Take a note for the amount of the filtered solution from corn milk sample that is used.

2.2 Accurate titration

Prepare the filtered solution from the preparation no. 1 and the Fehling solution similar to the procedure of the preliminary titration (point 2.1). Add the Fehling solution with the filtered solution from a burette. The amount of the added filtered solution should be 1-2 ml less than the amount needed to reach the end point of titration in the point 2.1. After the addition of the filtered solution, add the Fehling solution with 1-2 drops methylene blue indicator and quickly titrate the solution

against the filtered solution from the burette until the blue color of the Fehling solution disappeared.

Calculate the amount of reducing sugar in corn milk from the Table of Invert Sugar for 10 ml Fehling's Solution (D_1).

3. Analysis reducing sugar after inversion

Transfer 70 ml of the filtered solution from the preparation no. 1 into a 250 ml flask, and add with 10 ml of HCl 6.34 M. Warm the flask in a water bath that has been heated at 70 °C for 10 min and then cool it down immediately. After the solution in the flask is cool, adjust the pH of the solution to a pH value of 7.0 by adding NaOH 0.5 M. Transfer the whole solution to a 100 ml volumetric flask and adjust the volume of the solution to 100 ml by using distilled water. Mix the whole solution thoroughly. Transfer some of this solution into a 50 ml burette and use the solution to titrate the 10 ml mixed Fehling solution that is prepared according to the procedure point 2. Note the volume of the burette's solution that is needed to titrate the Fehling's solution. Calculate the amount of sugar in the corn milk samples after inversion from the Invert Sugar Table for 10 ml Fehling's solution (D_2)

$$\text{Reducing sugar} = D_1$$

$$\% \text{ sucrose} = (D_2 - D_1) \times 0.95$$

6. Moisture content (a method from an AOAC official Method no. 925.45, 2000)

Weight 3 g of corn milk powders into a moisture can with a tight-fit cover, which was known accurately the weight. Place loosely the covered can in a hot air oven at 100 °C. Dry the samples until the weight is constant (about 4 h). Press the cover tightly into the moisture can, then removed them from the oven, cool in an active dessicator and weight. Express the loss weight as a moisture content of the corn milk powders.

7. Fiber (a modified method from an AOAC official method no.978.10 (2000) and Rutjanakaikan (2001)

Determination**Apparatus**

1. Funnel for filtration of fiber

Reagents.

1. Petroleum ether (J.T. Baker, USA)
2. 0.12 M sulfuric acid solution (Merck, Germany)
3. 0.31 M sodium hydroxide solution (Merck, Germany)
4. 1 % HCl (Merck, Germany)
5. Diethyl ether (Merck, Germany)
6. Ethyl alcohol 95 % (Merck, Germany)

Procedure

Eight g of corn milk is extracted with petroleum ether and transfer into a 1000 ml flask. Add 200 ml of 0.12 M sulfuric acid solution into the flask and glass beads. Boil the solution and leave the solution boils exactly for 30 s. Prepare a funnel for filtration of fiber (using a whatman paper no. 41 that is known accurately its weight) by flowing boiling distilled water through the funnel to warm it. Then pour the acid solution that was boiled through the funnel and wash the solid that is collected on the funnel with boiled distilled water using vacuum. Wash the residue from the funnel in the flask that is used before with 200 ml of 0.31 M sodium hydroxide solution. Place the flask on a heater and boil for 30 min. Afterwards, pour the base solution that was boiled through a funnel using a whatman paper no. 41 that is known accurately for its weight and wash the solid on the funnel with boiled distilled water using vacuum. Wash the residues from the funnel into the flask that is used before with 30 ml 1 % of HCl. Next, wash the residues first with boiling distilled water and then 30 ml of 95 % ethyl alcohol 2 times and 30 ml diethyl ether 3 times. Transfer the residues into a crucible that is known accurately for its weight. Dry the crucible in a boiling-water bath and then take the crucible into a hot air oven at 100 °C until the weight is constant. Cool the crucible in an active desiccator and weight again. After that, take the crucible into an oven at 500 °C for 3 h. Cool the crucible in an active desiccator and weight. Calculate the percentage of fiber in corn milk samples.

8. Carotenoid in corn milk (a modified method from an AOAC official method no. 941.5, 2000)

A carotene standard curve

Reagents

1. Standard β -carotene (Fluka, Sigma-Aldrich, U.S.A)
2. Chloroform, AR Grade (Merck, Germany)
3. Hexane, AR Grade (Lab-scan, Ireland)
4. Acetone, AR Grade (Merck, Germany)

Procedure

1. Prepare a stock solution by dissolving 0.005 g β -carotene standard with 2.5 ml chloroform in a 50 ml beaker glass.
2. Adjust the volume of the solution with hexane in a 50 ml volumetric flask.
3. Dilute the stock solution by pipetting 5 ml of the solution into another 50 ml volumetric flask and then adjust the volume of the solution with hexane.
4. Pipette 1, 2, 3, 4, 5, 6, 7, 8 and 9 ml of the diluted solution into 10 ml volumetric flasks and adjust the volume with 10 % acetone in hexane.
5. Optimize the maximum wavelength by measuring the absorbance of the highest concentration of the standard solution with a spectrophotometer at 400-500 nm. Ten % acetone in hexane solution is used as a blank.
6. Adjust λ max at the spectrophotometer from the previous step and measure all the standard concentrations. The blank was 10 % acetone in hexane solution. Record all the result measurements.
7. A linear equation was drawn from all the standard solution results and a graph between β -carotene concentration (ppm) and absorbance values was produced. The method for preparing 10 % acetone in hexane solution was done by pipetting 10 ml acetone into a 100 ml volumetric flask and then adjust the volume with hexane.

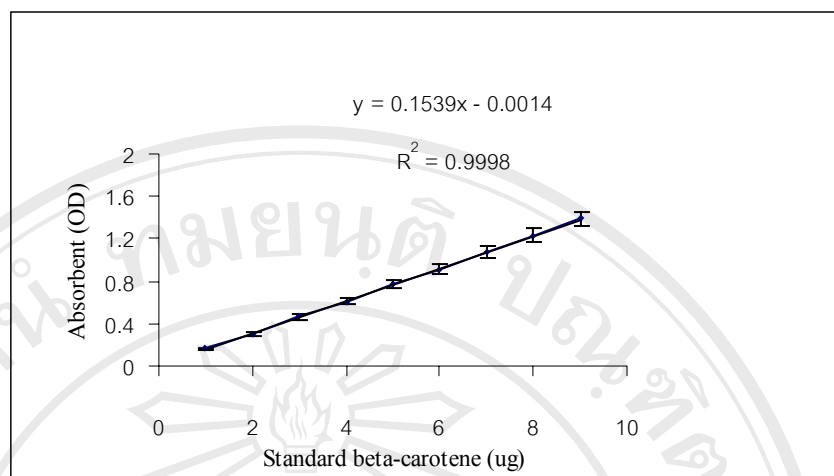


Figure C.1 β -carotene standard curve

The calculation to find out the amount of carotenoid in corn milk powder samples.

A correlation between the absorbance values and the β -carotene standard solution at $\lambda = 450 \text{ nm}$ was figured out in the Fig. C.1 and expressed in a linear equation which was:

$$Y = 0.1539x - 0.0014$$

where Y = absorbance values of carotenoid from corn milk powder samples.

X = the amounts of carotenoid in corn milk powder samples.

An extraction method of corn milk powders samples.

Five g of corn milk powders was put into a 250 volumetric flask. A 100 ml of 40 % acetone in hexane was poured into the flask which was then stirred with a magnetic stirrer for 10 min. The supernatant of the solution was separated from the corn milk powders by doing a filtration with a whatman filter paper no. 4 and collected in a 250 ml separated funnel. Whereas, the corn milk powders was washed for 2 times with 25 ml hexane. The solution from the washing steps was added into the separated funnel. The acetone in the separated funnel was removed by washing 5 times with 100 ml distilled water. Due to different solubility, the acetone which was dissolved in distilled water could be separated from the carotenoid which was dissolved in hexane by

using the separated funnel. The carotenoid which was dissolved in hexane was further filtered through a whatman filter paper no. 2 into a 250 ml beaker. The filtered solution was left in a hood until it was dried. After that the dried carotenoid was dissolved in 10 % acetone in hexane in a 50 ml volumetric flask. The mixed solution was then measured for its absorbance at a wavelength of 450 nm and the absorbance value was recorded.

For the blank which was 10 % acetone in hexane was measured 3 times for its absorbance.

Calculation the amount of carotenoid.

1000 ml diluted solution would have carotenoid = x mg.

50 ml diluted solution would have carotenoid = $(x/1000)*50$ mg.

Calculation for carotenoid in 5 g corn milk powders.

5 g corn milk powders would have carotenoid = z mg.

1 g corn milk powders would have carotenoid = $z/5$ mg.

The unit was changed from mg to μg by multiplying the result with 1000 to be used in report.

9. Vitamin C (a modified method from an AOAC official method no. 967.21, 2000 and Rattanapanon, 2001)

Reagents

9.1 An extraction solution (metaphosphoric acid-acetic acid solution)

Dissolve 15 g HPO_3 (Merck, Germany) in 40 ml CH_3COOH (Merck, Germany), add with 200 ml distilled water and further dilute to 500 ml with distilled water in a volumetric flask.

9.2 An ascorbic acid standard solution

Accurately weight 0.05 g ascorbic acid reference standard (Fisher chemicals, UK) that has been stored away from direct sunlight. Transfer the acid to a 50 ml volumetric flask. Dilute the acid to the volume of volumetric flask with distilled water immediately before use with HPO_3 - CH_3COOH solution.

9.3 Indophenol standard solution

Dissolve 0.05 g 2,6-dichloroindophenol (Merck, Germany) in 50 ml distilled water to which has been added with 42 mg NaHCO₃ (Merck, Germany) and then dilute to 200 ml with distilled water. Filter the solution through a fluted paper into an amber glass-stoppered bottle. Keep the bottle stoppered, out of direct sunlight and store in a refrigerator.

9.4 Vitamin C standard curve

Transfer 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml aliquots of ascorbic acid standard solution to each of 50 ml erlenmeyer flask and then dilute the solution to 7.0 ml with HPO₃-CH₃COOH solution. Titrate the mixed solution rapidly with indophenol solution from a 50 ml buret until light but distinct rose pink persists for ≥ 5 s. Each value represented a mean value of triplicate determinations on each sample.

Similarly titrate 3 blanks composed of 7.0 ml HPO₃-CH₃COOH solution plus volume of distilled water equal to ascorbic acid standard solution and titrated with indophenol solution in direct titration. Calculate the amount of vitamin C in corn milk powders from the vitamin C standard curve in Fig C. 2, according to the following equation:

$$y = 15.75x + 0.247$$

where, y = amount of indophenol standard solution

x = amount of vitamin C

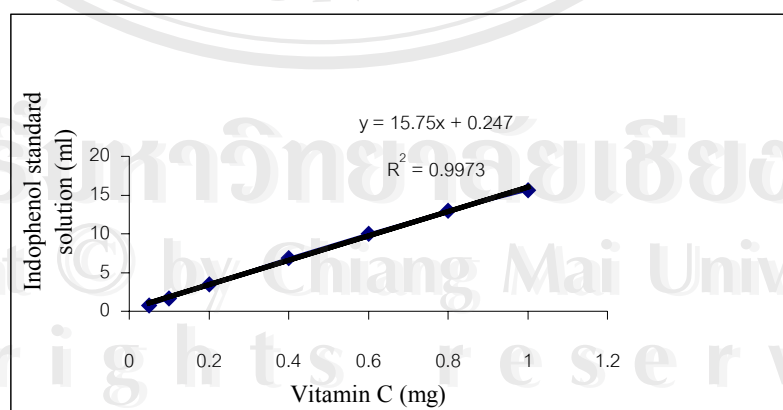
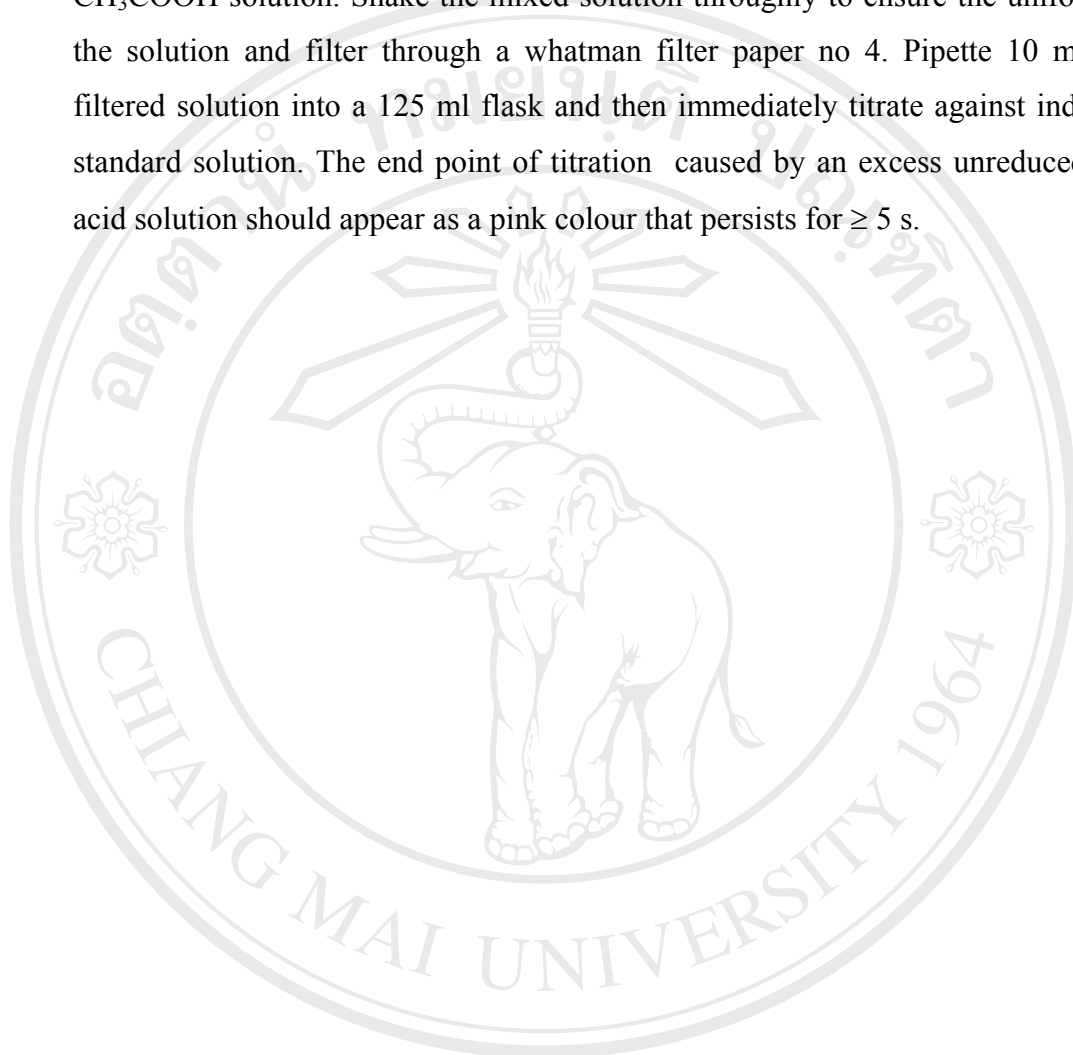


Figure C2 Vitamin C standard curve

9.5 Preparation of corn milk powder samples for vitamin C determination

Weight 20 g corn milk powder samples and mix with 100 ml $\text{HPO}_3\text{-CH}_3\text{COOH}$ solution. Shake the mixed solution thoroughly to ensure the uniformity of the solution and filter through a whatman filter paper no 4. Pipette 10 ml of the filtered solution into a 125 ml flask and then immediately titrate against indophenol standard solution. The end point of titration caused by an excess unreduced dye in acid solution should appear as a pink colour that persists for ≥ 5 s.



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Table B.1 Invert Sugar Table for 10 ml Fehling's Solution

ml of sugar solution required	Solution containing besides Invert sugar :									
	No sucrose		1 g sucrose per 100 ml		5 g sucrose per 100 ml		10 g sucrose per 100 ml		25 g sucrose per 100 ml	
	Invert sugar factor*	mg invert sugar per 100 ml	Invert Sugar Factor*	mg invert sugar per 100 ml	Invert sugar factor*	mg invert sugar per 100 ml	Invert sugar factor*	mg invert sugar per 100 ml	Invert sugar factor*	mg invert sugar per 100 ml
15	50.5	336	49.9	333	47.6	317	46.1	307	43.4	289
16	50.6	316	50.0	312	47.6	297	46.1	288	43.4	271
17	50.7	298	50.1	295	47.6	280	46.1	271	43.4	255
18	50.8	282	50.1	278	47.6	264	46.1	256	43.3	240
19	50.8	267	50.2	264	47.6	250	46.1	243	43.3	227
20	50.9	254.5	50.2	251.0	47.6	238.0	46.1	230.5	43.2	216
21	51.0	242.9	50.2	239.0	47.6	226.7	46.1	219.5	43.2	206
22	51.0	231.8	50.3	228.2	47.6	216.4	46.1	209.5	43.1	196
23	51.1	222.2	50.3	218.7	47.6	207.0	46.1	200.4	43.0	187
24	51.2	213.3	50.3	209.8	47.6	198.3	46.1	192.1	42.9	179
25	51.2	204.9	50.4	201.6	47.6	190.4	46.0	184.0	42.8	171
26	51.3	197.4	50.4	193.8	47.6	183.1	46.0	176.9	42.8	164
27	51.4	190.4	50.4	186.7	47.6	176.4	46.0	170.4	42.7	158
28	51.4	183.7	50.5	180.2	47.7	170.3	46.0	164.3	42.7	152
29	51.5	177.6	50.5	174.1	47.7	164.5	46.0	158.6	42.6	147
30	51.5	171.7	50.5	168.3	47.7	159.0	46.0	153.3	42.5	142
31	51.6	166.3	50.6	163.1	47.7	153.9	45.9	148.1	42.5	137
32	51.6	161.2	50.6	158.1	47.7	149.1	45.9	143.4	42.4	132
33	51.7	156.6	50.6	153.3	47.7	144.5	45.9	139.1	42.3	128
34	51.7	152.2	50.6	148.9	47.7	140.3	45.8	134.9	42.2	124
35	51.8	147.9	50.7	144.7	47.7	136.3	45.8	130.9	42.2	121
36	51.8	143.9	50.7	140.7	47.7	132.5	45.8	127.1	42.1	117
37	51.9	140.2	50.7	137.0	47.7	128.9	45.7	123.5	42.0	114
38	51.9	136.6	50.7	133.5	47.7	125.5	45.7	120.3	42.0	111
39	52.0	133.3	50.8	130.2	47.7	122.3	45.7	117.1	41.9	107
40	52.0	130.1	50.8	127.0	47.7	119.2	45.6	114.1	41.8	104
41	52.1	127.1	50.8	123.9	47.7	116.3	45.6	111.2	41.8	102
42	52.1	124.2	50.8	121.0	47.7	113.5	45.6	108.5	41.7	99
43	52.2	121.4	50.8	118.2	47.7	110.9	45.5	105.8	41.6	97
44	52.2	118.7	50.9	115.6	47.7	108.4	45.5	103.4	41.5	94
45	52.3	116.1	50.9	113.1	47.7	106.0	45.4	101.0	41.4	92
46	52.3	113.7	50.9	110.6	47.7	103.7	45.4	98.7	41.4	90
47	52.4	111.4	50.9	108.2	47.7	101.5	45.3	96.4	41.3	88
48	52.4	109.2	50.9	106.0	47.7	99.4	45.3	94.3	41.2	86
49	52.5	107.1	51.0	104.0	47.7	97.4	45.2	92.3	41.1	84
50	52.5	105.1	51.0	102.0	47.7	95.4	45.2	90.4	41.0	82

*mg of invert sugar corresponding to 10 ml of Fehling's solution.

Appendix C

Microbiological quality analysis

1. Total plate counts (Robert *et al.*, 1995)

1.1 Equipment

1. Petri dishes
2. Test tubes
3. Pipettes
4. A waterbath model 4999 (Mettler, Germany)
5. A hot air oven model UM 500 (Mettler, Germany)
6. An incubator (Gallenkamp, England)
7. An autoclave (Gallenkamp, England)

1.2 Chemical

1. Peptone (Merck, Germany)
2. Plate Count Agar (PCA) (Charlau Chemie S. A. Barcelona, Spain).

1.3 Preparation of culture media and reagents

1. Peptone diluent. Dissolved 0.1 g peptone in 100 ml distilled water and then autoclaving for 15 min at 121 °C.
2. PCA. Dissolved 23.5 g PCA in 1 l distilled water and then autoclaving for 15 min at 121 °C.

1.4 Measurement of total microorganisms in corn milk powder samples

1. Prepare 1:10, 1:100, 1:1000 and 1:10000 dilution of the corn milk powder samples. Mix the samples thoroughly by shaking to ensure uniformity of the solution.
2. Pipette 1 ml representative samples from each dilution into a petri dish. Two petri dishes for each dilution.
3. Pour 15-20 ml PCA into a petri dish from 2 and mix the sample and the media thoroughly by gently shaking into the left and right combined with up and down. Let the petri dishes stand on a bench until the PCA media was harden with a smooth even surface on the top.
4. Incubate invertly the petri dishes for 48 h at 35 °C. Count all colonies that growth in the petri dishes in the range of 30-300 colonies. The average

number of colonies from duplicate plates for each dilution was calculated and used in the report.

2. Yeast and mold counts (Robert *et al.*, 1995)

2.1 Equipment

1. Petri dishes
2. Test tubes
3. Pipettes
4. A waterbath model 4999 (Mettler, Germany)
5. A hot air oven model UM 500 (Mettler, Germany)
6. An incubator (Gallenkamp, England)
7. An autoclave (Gallenkamp, England)

2.2 Chemical

1. Peptone (Merck, Germany)
2. Potato Dextrose Agar (PDA) (Charlau Chemie S. A. Barcelona, Spain)

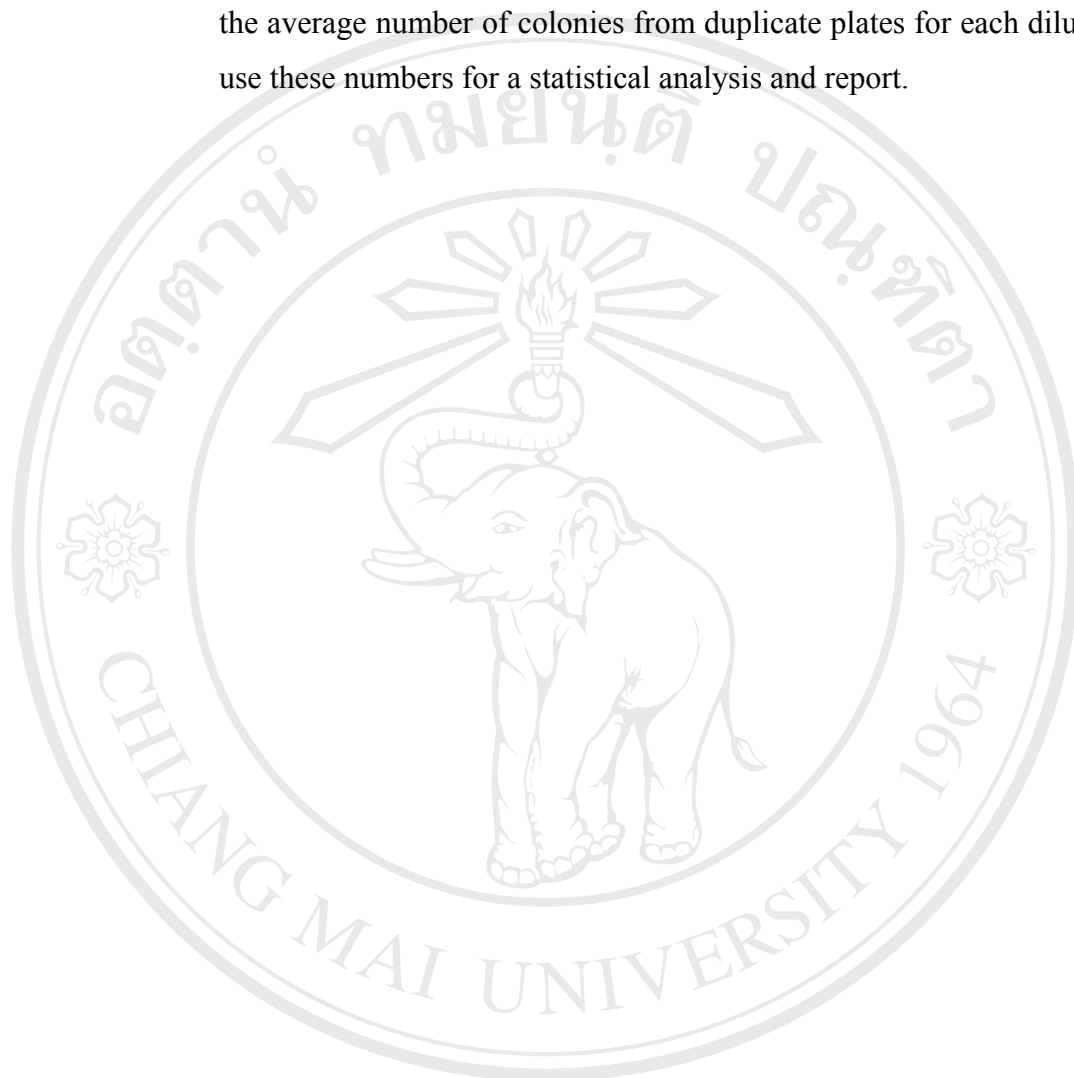
2.3 Preparation of culture media and reagents

1. Peptone diluent. Dissolved 0.1 g peptone in 100 ml distilled water and then autoclaving for 15 min at 121 °C.
2. PDA. Dissolved 39.0 g PDA in 1 l distilled water and then autoclaving for 15 min at 121 °C.

2.4 Measurement of yeast and mold in corn milk powder samples

1. Prepare 1:10, 1:100, 1:1000 and 1:10000 dilution of the corn milk powder samples. Mix the samples thoroughly by shaking to ensure uniformity of the solution.
2. Pipette 1 ml representative samples from each dilution into a petri dish. Two petri dish for each dilution.
3. Pour 15-20 ml PDA into a petri dish from 2 and mix the sample and the media thoroughly by gently shaking into the left and right combined with up and down. Let the petri dishes stand on a bench until the PDA media was harden with a smooth even surface on the top.

4. Incubate invertly the petri dishes for 120 h at 25 °C. Count all colonies that growth in the petri dishes within the range of 30-300 colonies. Calculate the average number of colonies from duplicate plates for each dilution and use these numbers for a statistical analysis and report.



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