## APPENDIX A

### Reducing Sugar Determination by DNS Method (Miller, 1959)

**Chemical Reagents**

DNS reagent contained 10 g/L 3,5-dinitrosalicylic acid (DNS), 0.5 g/L ($Na_2SO_3$), 182 g/L sodium potassium tartrate (Na-K tartrate), 10 g/L (NaOH), 2 g/L phenol and 1 L deionized water.

**DNS Solution Preparation**

First, NaOH was dissolved in 700 mL of deionized water. Then, Na-K tartrate was added during stir continuously. After that, DNS was added during stir continuously. After all DNS is well dissolve, $Na_2SO_3$ and phenol were added, respectively. Finally, the solution was adjusted to final volume of 1 L with volumetric flask. DNS solution is kept in brown glass bottle.

**Residual Sucrose Determination Procedure**

After treated sample with 2N HCL and neutralized, 1 mL of sample was mixed with 1 mL DNS solution and boiled for 10 min. Then, the sample was cooled down by immersing into cold water immediately. After that, 5 mL water was added and mixed well. Finally, the absorbance of sample was measured at 540 nm. The absorbance value was converted to sucrose concentration with standard curve (Fig. A1).

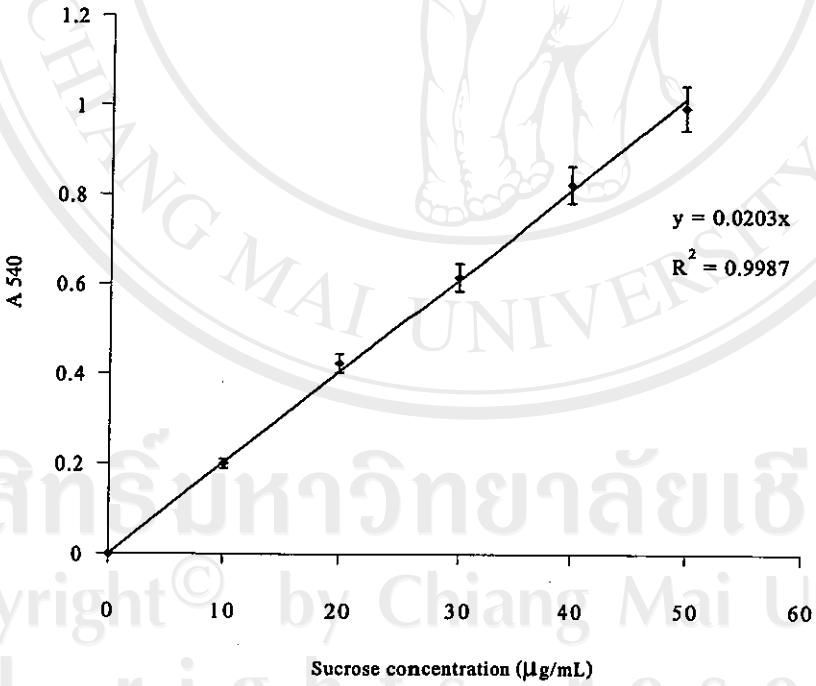


**Fig. A1** Standard curve of sucrose concentration

## APPENDIX B

## ANOVA ANALYSIS

**Appendix B-1.** Statistic analysis of the effect of moist-air supplement for EPS production on MRS agar plate culture

| SOURCE | DF | SS | MS | F | Prob > F |
|---|---|---|---|---|---|
| TRT (A) | 1 | 6.89082 | 6.89082 | 9.615 | 0.0001 |
| REP (B) | 2 | 0.07543 | 0.03772 | 52.63 | 0.0186 |
| A*B | 2 | 0.00143 | 7.167E-04 | | |
| TOTAL | 5 | 6.96768 | | | |

C.V. 1.05 R-Squared 0.9852

TUKEY (HSD) COMPARISON OF MEANS OF EPS BY TRT

| TRT | MEAN |
|---|---|
| air supplement | 7.7600[a] |
| non-air supplement | 5.6517[b] |

**Appendix B-2.** Statistic analysis of the effect of initial sucrose concentration for EPS production on MRS agar plate culture

| SOURCE | DF | SS | MS | F | Prob > F |
|---|---|---|---|---|---|
| TRT (A) | 6 | 19.2129 | 3.20214 | 145.55 | 0.0000 |
| REP (B) | 2 | 0.01137 | 0.00568 | 0.26 | 0.7765 |
| A*B | 12 | 0.26400 | 0.02200 | | |
| TOTAL | 20 | 19.4882 | | | |

C.V. 0.31 R-Squared 0.9998

TUKEY (HSD) COMPARISON OF MEANS OF EPS BY TRT

| TRT | MEAN |
|---|---|
| 12 g/L | 8.76[a] |
| 18 g/L | 8.32[a] |
| 20 g/L | 8.30[b] |
| 15 g/L | 8.28[b] |
| 9 g/L | 7.76[bc] |
| 6 g/L | 6.59[c] |
| 3 g/L | 4.48[d] |

**Appendix B-3**. Statistic analysis of the effect of rice husk to MRS medium ratio for EPS production on rice husk culture

| SOURCE | DF | SS | MS | F | Prob > F |
|---|---|---|---|---|---|
| TRT (A) | 4 | 138.172 | 34.5431 | 1015.28 | 0.0000 |
| REP (B) | 2 | 0.13301 | 0.06650 | 1.95 | 0.2036 |
| A*B | 8 | 0.27219 | 0.03402 | | |
| TOTAL | 14 | 138.578 | | | |

C.V. 3.50 R-Squared 0.9972

TUKEY (HSD) COMPARISON OF MEANS OF EPS BY TRT

| TRT | MEAN |
|---|---|
| 1:5 | 8.857[a] |
| 1:4 | 8.716[a] |
| 1:3 | 8.643[a] |
| 1:2 | 5.008[b] |
| 1:1 | 1.116[c] |

**Appendix B-4.** Statistic analysis of the effect of inoculum size for EPS production on rice husk culture

| SOURCE | DF | SS | MS | F | Prob > F |
|---|---|---|---|---|---|
| TRT (A) | 6 | 4.42691 | 0.73782 | 55.03 | 0.0000 |
| REP (B) | 2 | 0.01631 | 0.00816 | 0.61 | 0.5602 |
| A*B | 12 | 0.16088 | 0.01341 | | |
| TOTAL | 20 | 4.60410 | | | |

C.V. 1.23 R-Squared 0.9608

TUKEY (HSD) COMPARISON OF MEANS OF EPS BY TRT

| TRT | MEAN |
|---|---|
| 15% | $9.86^{a}$ |
| 12.5% | $9.66^{b}$ |
| 10% | $9.29^{bc}$ |
| 7.5% | $9.28^{bc}$ |
| 25% | $8.93^{c}$ |
| 20% | $8.66^{d}$ |
| 2.5% | $8.35^{d}$ |

**Appendix B-5.** Statistic analysis of the effect of moist-aeration supplement for EPS production on rice husk culture

| SOURCE | DF | SS | MS | F | Prob > F |
|---|---|---|---|---|---|
| TRT (A) | 1 | 0.50460 | 0.50460 | 15.50 | 0.0589 |
| REP (B) | 2 | 0.02710 | 0.01355 | 0.42 | 0.7061 |
| A*B | 2 | 0.06510 | 0.03255 | | |
| TOTAL | 5 | 0.59680 | | | |
| C.V. 3.10 | | R-Squared | 0.6232 | | |

**Appendix B-6.** Statistic analysis of the effect of initial sucrose concentration for EPS production on rice husk culture

| SOURCE | DF | SS | MS | F | Prob > F |
|---|---|---|---|---|---|
| TRT (A) | 9 | 11.1353 | 1.23726 | 81.17 | 0.0000 |
| REP (B) | 2 | 0.08936 | 0.04468 | 2.93 | 0.0791 |
| A*B | 18 | 0.27437 | 0.01524 | | |
| TOTAL | 29 | 11.4991 | | | |

C.V. 1.07 R-Squared 0.9874

TUKEY (HSD) COMPARISON OF MEANS OF EPS BY TRT

| TRT | MEAN |
|---|---|
| 20 g/L | $10.01^{a}$ |
| 25 g/L | $9.89^{a}$ |
| 60 g/L | $9.46^{b}$ |
| 50 g/L | $9.37^{bc}$ |
| 30 g/L | $9.30^{bc}$ |
| 80 g/L | $9.16^{bc}$ |
| 10 g/L | $9.15^{bc}$ |
| 15 g/L | $9.02^{c}$ |
| 70 g/L | $9.01^{c}$ |
| 40 g/L | $7.63^{d}$ |

## APPENDIX C

**ANOVA for response surface quadratic model of CCD for optimize moist-air flowrate, MRS agar medium volume and incubation time on EPS production under agar plate culture**

**Analysis of variance table [Partial sum of squares]**

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model | 313.09 | 9 | 34.79 | 0.99 | 0.5000 |
| *A* | *8.60* | *1* | *8.60* | *0.25* | *0.6311* |
| *B* | *148.06* | *1* | *148.06* | *4.22* | *0.0669* |
| *C* | *2.08* | *1* | *2.08* | *0.059* | *0.8126* |
| $A^2$ | *1.28* | *1* | *1.28* | *0.036* | *0.8524* |
| $B^2$ | *130.64* | *1* | *130.64* | *3.73* | *0.0824* |
| $C^2$ | *12.10* | *1* | *12.10* | *0.35* | *0.5700* |
| *AB* | *7.70* | *1* | *7.70* | *0.22* | *0.6493* |
| *AC* | *2.10* | *1* | *2.10* | *0.060* | *0.8116* |
| *BC* | *5.88* | *1* | *5.88* | *0.17* | *0.6908* |
| Residual | 350.56 | 10 | 35.06 | | |
| *Lack of Fit* | *293.29* | *5* | *58.66* | *5.12* | *0.0487* |
| *Pure Error* | *57.27* | *5* | *11.45* | | |
| Cor Total | 663.65 | 19 | | | |

| | | | |
|---|---|---|---|
| Std. Dev. | 5.92 | R-Squared | 0.9418 |
| Mean | 7.07 | Adj R-Squared | -0.0036 |
| C.V. | 83.69 | Pred R-Squared | -2.6329 |
| PRESS | 2410.99 | Adeq Precision | 4.033 |

**Final Equation in Terms of Code Factors:**

Response 3 (EPS) =

$$
\begin{aligned}
&+4.23 \\
&+0.81 * A \\
&-3.27 * B \\
&+0.39 * C \\
&+0.31 * A^2 \\
&+2.92 * B^2 \\
&+0.93 * C^2 \\
&+0.98 * A * B \\
&-0.51 * A * C \\
&+0.86 * B * C
\end{aligned}
$$

Where A, B and C are MRS agar volume, incubation time and moist-air flowrate, respectively.

**The prediction from the optimum conditions for EPS production**

| Factor | Name | Level | Low Level | High Level | Std. Dev. |
|---|---|---|---|---|---|
| A | medium | 10.00 | 10.00 | 18.00 | 0.000 |
| B | time | 24.00 | 17.00 | 31.00 | 0.000 |
| C | air | 28.00 | 7.00 | 28.00 | 0.000 |

| | Prediction | SE Mean | 95% CI low | 95% CI high | SE Pred | 95% PI low | 95% PI high |
|---|---|---|---|---|---|---|---|
| **EPS** | 8.24402 | 0.83 | 6.39 | 10.10 | 1.61 | 4.66 | 11.83 |
| **Sucrose** | 2.24938 | 0.44 | 1.28 | 3.22 | 0.84 | 0.37 | 4.13 |
| **Cell** | 5.56609 | 3.59 | -2.42 | 13.56 | 6.92 | -9.86 | 20.99 |

## APPENDIX D

**ANOVA for mixture quadratic model for optimize nitrogen sources concentration on EPS production on rice husk support culture**

**Analysis of variance table [Partial sum of squares]**

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model | 17.67 | 9 | 1.96 | 3.29 | 0.0387 |
| *Linear Mixture* | *3.25* | *3* | *1.08* | *1.81* | *0.2081* |
| *AB* | *0.024* | *1* | *0.024* | *0.041* | *0.8435* |
| *AC* | *3.603E-003* | *1* | *3.603E-003* | *6.039E-003* | *0.9396* |
| *AD* | *3.53* | *1* | *3.53* | *5.92* | *0.0352* |
| *BC* | *4.86* | *1* | *4.86* | *8.15* | *0.0171* |
| *BD* | *3.95* | *1* | *3.95* | *6.62* | *0.0277* |
| *CD* | *2.05* | *1* | *2.05* | *3.43* | *0.0937* |
| Residual | 5.97 | 10 | 0.60 | | |
| *Lack of Fit* | *4.53* | *5* | *0.91* | *3.16* | *0.1164* |
| *Pure Error* | *1.44* | *5* | *0.29* | | |
| Cor Total | 23.64 | 19 | | | |

| | | | |
|---|---|---|---|
| Std. Dev. | 0.77 | R-Squared | 0.9776 |
| Mean | 8.63 | Adj R-Squared | 0.5204 |
| C.V. | 8.96 | Pred R-Squared | -0.7208 |
| PRESS | 40.68 | Adeq Precision | 7.826 |

**Final Equation in Terms of Pseudo Components:**

| | |
|---|---|
| EPS | = |
| +8.21 | * A |
| +6.15 | * B |
| +8.71 | * C |
| -19.83 | * D |
| +1.03 | * A * B |
| +0.31 | * A * C |
| +42.02 | * A * D |
| +13.57 | * B * C |
| +48.50 | * B * D |
| +33.01 | * C * D |

Where A, B, C and D are the concentration of yeast extract, meat extract, bacto-peptone and diammonium hydrogen citrate, respectively.

**The prediction from the optimum conditions for EPS production**

| Component | Name | Level | Low Level | High Level | Std. Dev. |
|---|---|---|---|---|---|
| A | yeast ext | 0.43 | 0.000 | 1.00 | 0.000 |
| B | meat ext | 0.36 | 0.000 | 1.00 | 0.000 |
| C | peptone | 0.50 | 0.000 | 1.50 | 0.000 |
| D | am. citrate | 0.21 | 0.000 | 0.50 | 0.000 |
| | Total = | 1.50 | | | |

| | Prediction | SE Mean | 95% CI low | 95% CI high | SE Pred | 95% PI low | 95% PI high |
|---|---|---|---|---|---|---|---|
| **EPS** | 10.30152 | 0.51 | 8.85 | 11.14 | 0.93 | 7.92 | 12.06 |
| **Sucrose** | 3.98456 | 0.18 | 3.60 | 4.37 | 0.80 | 2.28 | 5.68 |
| **Cell** | 1.696 | 0.10 | 1.47 | 1.92 | 0.47 | 0.71 | 2.68 |

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- Seminar presentation at the Department of Biotechnology, Faculty of Agro-Industry, Chiang Mai University. In the topic of "Effect of Some Factors on Exopolysaccharide Production from *Pediococcus urinae-equi* Cultured on Solid Supports'' 31st January – 1st February 2003.

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- Participation in "A seminar on Fermentation Technology : Current and Future Directions of Biotechnology in Waste Management for Sustainable Development'' at Novotel Hotel, Chiang Mai, Thailand. 13th-15th August 2002.

**Publication**

- Maungtoom, K., Seesuriyachan, P., Wongroung, S., Techapan, C. and Hanmoungjai, P. (2002). Screening of Exopolysaccharide-Producing Lactic Acid Bacteria Strains from Traditional Thai Pork Sausage. *Proceeding of the 14 th Annual Meeting of the Thai Society for Biotechnology, 12th -15th November 2002*, Khon Kaen, Thailand.

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