

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่

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## APPENDIX A

### Preparation of the medium for fungi and chemical reagents

#### A1. Preparation of the medium for fungi

##### Potato dextrose agar (PDA)

Glucose	20.0 g
Agar	15.0 g
Potato, infusion form	1000 ml

##### Preparation of potato, infusion form

- Peel and dice potatoes
- Add 300 g of potato to 500 ml of distilled water
- Gently heat and bring to boiling
- Continued boiling for 30 minutes
- Filter through gauze, Bring volume of the filtrate to 1000 ml.

##### Preparation of medium

- Add glucose and agar to 1000 ml of potato infusion
- Mix, gently heat and bring to boiling
- Autoclave for 15 minutes at 15 psi pressure at 121 °C

**Rose Bengal agar**

Agar	15.0 g
Glucose	10.0 g
Malt extract agar	20.0 g
Rose Bengal	0.05 g
Chloramphenicol	10.0 ml

**Preparation of chloramphenicol solution**

- Add 0.10 g of chloramphenicol to distilled water and bring volume to 10.0 ml. Mix thoroughly
- Filter and sterilize

**Preparation of medium**

- Add components, except chloramphenicol solution, to distilled water and bring the volume to 990 ml
- Mix thoroughly
- Gently heat and bring to boiling
- Autoclave for 15 minutes at 15 psi pressure at 121 °C
- Cool to 45 °C aseptically add sterile the chloramphenicol solution
- Mix thoroughly
- Pour into sterile Petri dishes.

## A2. Preparation of the chemical reagents for determination of the total antioxidant capacity

### 1. 0.2 mg/ml Standard gallic acid solution

The gallic acid 0.0200 g was dissolved and made up to 100 ml

### 2. Reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate)

10.64 g sodium phosphate and 4.94 g ammonium molybdate was dissolved and transferred to 1000 ml volume flask. 33.70 ml concentrated sulphuric acid was added and the volume was adjusted to 1000 ml.

**Table A1.1** The volume of chemical reagents for standard total antioxidant capacity graph

Solutions	Volume (ml)					
	1	2	3	4	5	6
0.2 mg/ml gallic acid	0.05	0.10	0.15	0.20	0.25	0.30
Distilled water	0.95	0.90	0.85	0.80	0.75	0.70
Reagent solution	3.0	3.0	3.0	3.0	3.0	3.0
Stirring						
incubated at 95 °C for 90 minutes						
Measurement the absorbance at 725 nm						

### **A3. Preparation of the chemicals reagent for $\beta$ -carotene bleaching activity**

#### **$\beta$ -carotene emulsion**

0.0040 g  $\beta$ -carotene was dissolved in 10 ml chloroform. 200  $\mu$ l linoleic acid and 2 ml Tween 20 were added. Removed chloroform with nitrogen gas for 5 minutes and immediately made up to 1000 ml with oxygenated water.

#### **A3.1 Preparation of standard calibration curve for $\beta$ -carotene bleaching activity of standard BHT**

0.05 mg/ml standard BHT solution was diluted in the different concentrations with methanol. The reagent solution was added to test tubes as shown in the following Table A1.2. The tubes were incubated at 50°C for 2 hours. After that, the mixtures were shaken; the absorbance of the solutions were measured at 470 nm.

#### **A3.2 $\beta$ -carotene bleaching activity of the samples**

The extract of the samples were taken in a test tube and made up to 0.2 ml with methanol. The samples were combined with  $\beta$ -carotene solution emulsion. The absorbance at 470 nm was determined after incubated the test tubes at 50°C for 2 hours. The percent inhibition activity was calculated.

The percent inhibition of all experiment was calculated and plot between % inhibition of  $\beta$ -carotene oxidation versus concentration of standard or the samples to find IC<sub>50</sub> values (concentration of sample required to scavenge 50% of free radicals)

**Table A1.2** The volume of chemical reagents for  $\beta$ -carotene bleaching activity of standard BHT

Solutions	Volume (ml)						
	1	2	3	4	5	6	7
0.05 mg/ml standard BHT	0.01	0.02	0.03	0.04	0.05	0.06	0.07
MeOH	0.19	0.18	0.17	0.16	0.15	0.14	0.13
Stirring							
$\beta$ -carotene solution emulsion	5.0	5.0	5.0	5.0	5.0	5.0	5.0
incubated at 50°C for 2 h							
Measurement at 470 nm							

## APPENDIX B

### Calculation of the data

#### B1. Percentage yield of red yeast rice

Percentage yield of red yeast rice =  $\frac{\text{Weight of red yeast rice (g)}}{\text{Weight of stream rice}} \times 100$

Weight of stream rice

e.g. Percentage yield of Mali105 =  $\frac{16.39 \text{ g}}{100 \text{ g}} \times 100$

100 g

Percentage yield of Mali105 = 16.39 %

#### B2. Measuring the content red pigment of red yeast rice

Fermented rice (red yeast rice), 0.5 g in 50 ml 75 % ethanol extract or the sample concentration of 10 mg/ml was used. The determination of the content of red pigment was done by measuring the absorbance at 500 nm after dilution of the extract to the suitable concentration for the spectrophotometer.

**Table B1.1** The content absorbance of red yeast rice at 500 nm

<b>Fermented rice</b>	<b>DF</b>	<b>AU</b>	<b>DFxAU</b>	<b>AU/g</b>
Comryr	7	0.37994	2.65958	5.31916
Mali105 <sup>a</sup> 2 weeks	5	0.45147	2.25735	4.5147
Mali105 <sup>a</sup> 3 weeks	1	0.71020	0.71020	1.4204
Mali105 <sup>b</sup> 2 weeks	1	0.45749	0.45749	0.91498
Mali105 <sup>b</sup> 2 weeks	5	0.36014	1.8007	3.6014
Kam <sup>a</sup> 2 weeks	5	0.36302	1.8151	3.6302
Kam <sup>a</sup> 3 weeks	5	0.60645	3.03225	6.0645
Kam <sup>b</sup> 2 weeks	5	0.69921	3.49605	6.9921
Kam <sup>b</sup> 3 weeks	5	0.50613	2.53065	5.0613
RD6 <sup>a</sup> 2 weeks	50	0.30113	15.0565	30.113
RD6 <sup>a</sup> 3 weeks	50	0.39902	19.951	39.902
RD6 <sup>b</sup> 2 weeks	50	0.45039	22.5195	45.039
RD6 <sup>b</sup> 3 weeks	50	0.42160	21.0800	42.160
SPT1 <sup>a</sup> 2 weeks	50	0.29232	14.616	29.232
SPT1 <sup>a</sup> 3 weeks	50	0.34129	17.0645	34.129
SPT1 <sup>b</sup> 2 weeks	50	0.36094	18.047	36.094
SPT1 <sup>b</sup> 3 weeks	50	0.42697	21.3485	42.697

<sup>a</sup> Fermented without soybean milk, <sup>b</sup> Fermented with soybean milk

AU = absorbance at 500 nm.

DF = Dilution factor

AU/g =  $\frac{\text{AU} \times \text{DF}}{\text{Weight of fermented rice (0.5 g)}}$

Weight of fermented rice (0.5 g)

### B3. Measuring the content of compactin, monacolin K content

Fermented rice (red yeast rice), 0.5 g in 50 ml 75 % ethanol extract was used. Determination of the content of compactin and monacolin K were carried out by comparing the peak area with the peak area of the standard reference compounds obtained by HPLC at the same condition. The calculation was done as follows.

$$\% \text{Content} = \frac{\text{concentration of standard} \times \text{Peak area (ryr)}}{\text{Peak area of standard reference compound}} \times 100$$

Peak area of standard reference compound

ryr = fermented rice or red yeast rice

Concentration of standard compactin is 0.05 mg/ml

Concentration of standard monacolin K is 0.02 mg/ml

Example Mali105 3 weeks without soy bean milk

Peak area of standard monacolin is 126.4558 and the concentration of monacolin is 0.02 mg/ml

Peak area of Mali105 is 22.7200, therefore

$$\text{Monacolin content is } \frac{0.02 \times 22.7200}{126.4558} = 0.00359 \text{ mg/ml}$$

Extract of Mali105 1 ml has monacolin K = 0.00359 mg

Extract of Mali105 50 ml has monacolin K =  $0.00359 \times 50 = 0.1795$  mg

Mali105 0.5 g has monacolin K = 0.1795 mg

Mali105 1.0 g has monacolin K =  $\frac{0.1795}{0.5} = 0.359$  mg

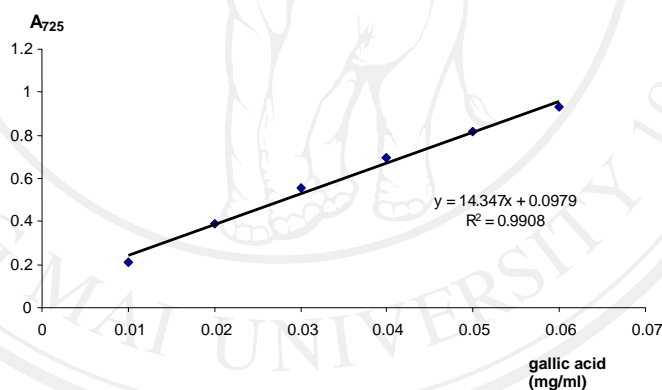
0.5

Mali105 3 weeks without soybean milk has monacolin K content = 0.359 mg/g

#### B4. Antioxidant capacity

Standard antioxidant capacity preparation of standard calibration curve for total antioxidant capacity was carried out using standard gallic acid. 0.2 mg/ml Gallic acid solution was prepared and diluted to give the different concentrations (0.01, 0.02, 0.03, 0.04, 0.05, 0.06 mg/ml). 3 ml of the reagent solution was added. The tubes were incubated at 95 ° C for 90 min. After cooling the absorbance was measured at 725 nm.

To determine the total antioxidant capacity of the extracts, the samples were placed in test tubes and made up to 1 ml with distilled water. The tubes were incubated at 95 ° C for 90 minutes and after cooling, the absorbance was measured at 725 nm.



**Figure B1.1** Show as the linear equation from standard calibration curve for antioxidant capacity was

$$Y = 14.347X + 0.0979$$

where X is GAE in the sample solution made up to 1 ml and Y is the absorbance. The GAE of the original sample solution(C) is therefore calculated from

$$C = X \times 1 / V$$

where V is the volume of the sample used.

**Table B1.2** The antioxidant capacity in the samples.

<b>Fermented rice</b>	<b>V(ml)</b>	<b>A<sub>1</sub></b>	<b>A<sub>2</sub></b>	<b>GAE(mg/ml)</b>	<b>GAE(mg/ml)</b>	<b>aver(mg/ml)<math>\pm</math>SD</b>
Commercial red yeast rice	0.2	0.34	0.351	0.084373	0.088207	0.08629 $\pm$ 0.002711
Mali105	1	0.315	0.32	0.015132	0.015481	0.015306 $\pm$ 0.000246
Mali105 <sup>a</sup> 2 weeks	0.2	0.571	0.587	0.164878	0.170454	0.167666 $\pm$ 0.003943
Mali105 <sup>a</sup> 3 weeks	0.2	0.459	0.426	0.125845	0.114344	0.120095 $\pm$ 0.008132
Mali105 <sup>b</sup> 2 weeks	0.2	0.856	0.83	0.264202	0.25514	0.259671 $\pm$ 0.006407
Mali105 <sup>b</sup> 3 weeks	0.2	0.512	0.523	0.144316	0.148149	0.146233 $\pm$ 0.002711
Kam	0.5	0.481	0.467	0.053405	0.051453	0.052429 $\pm$ 0.00138
Kam <sup>a</sup> 2 weeks	0.2	0.511	0.534	0.143967	0.151983	0.147975 $\pm$ 0.005668
Kam <sup>a</sup> 3 weeks	0.2	0.601	0.609	0.175333	0.178121	0.176727 $\pm$ 0.001971
Kam <sup>b</sup> 2 weeks	0.2	0.702	0.731	0.210532	0.220638	0.215585 $\pm$ 0.007146
Kam <sup>b</sup> 3 weeks	0.2	0.651	0.655	0.192758	0.194152	0.193455 $\pm$ 0.000986

<b>Fermented rice</b>	<b>V(ml)</b>	<b>A<sub>1</sub></b>	<b>A<sub>2</sub></b>	<b>GAE(mg)</b>	<b>GAE(mg)</b>	<b>aver(mg)<sub>±</sub>SD</b>
RD6	0.7	0.231	0.245	0.013253	0.014647	0.01395 <sub>±</sub> 0.000986
RD6 <sup>a</sup> 2 weeks	0.1	0.595	0.619	0.346484	0.363212	0.354848 <sub>±</sub> 0.011829
RD6 <sup>a</sup> 3 weeks	0.1	0.807	0.847	0.49425	0.52213	0.50819 <sub>±</sub> 0.019714
RD <sup>b</sup> 2weeks	0.1	0.805	0.831	0.492856	0.510978	0.501917 <sub>±</sub> 0.012814
RD6 <sup>b</sup> 3 weeks	0.1	0.858	0.843	0.529797	0.519342	0.52457 <sub>±</sub> 0.007393
SPT1	1	0.345	0.366	0.017223	0.018687	0.017955 <sub>±</sub> 0.001035
SPT1 2 weeks	0.1	0.623	0.601	0.366	0.350666	0.358333 <sub>±</sub> 0.010843
SPT1 <sup>a</sup> 3 weeks	0.1	0.696	0.743	0.416882	0.449641	0.433261 <sub>±</sub> 0.023164
SPT1 <sup>b</sup> 2 weeks	0.1	0.784	0.775	0.478218	0.471945	0.475082 <sub>±</sub> 0.004436
SPT1 <sup>b</sup> 3 weeks	0.1	0.878	0.851	0.543737	0.524918	0.534328 <sub>±</sub> 0.013307

**GAE= Gallic acid equivalent**

A<sub>1</sub> absorbance 1<sup>st</sup> time, A<sub>2</sub> absorbance 2<sup>nd</sup> time

<sup>a</sup> Fermented without soybean milk, <sup>b</sup> Fermented with soybean milk

### B5. $\beta$ -carotene bleaching activity

The inhibition percentage for  $\beta$ -carotene oxidation by the sample were determined as shown below.

The absorbance at 470 nm was for example 0.7044 (t=0) and 0.1432 (t=120) while the absorbance of the blank solution was 0.6277 (t=0) and 0.0316 (t=120). The total volume of each tube was 5.2 ml.

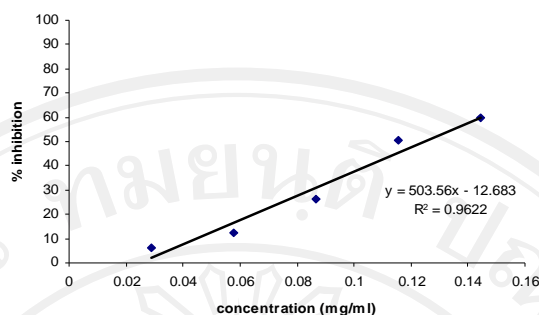
The percentage of the inhibition was calculated from the equation below.

$$\begin{aligned} \text{Inhibition percentage for } \beta\text{-carotene oxidation} &= \left\{ 1 - \frac{(A_0 - A_t)}{(A_0^0 - A_t^0)} \right\} \times 100 \\ &= \left\{ 1 - \frac{(0.7044 - 0.1432)}{(0.6277 - 0.0316)} \right\} \times 100 \end{aligned}$$

$$\text{Inhibition percentage} = 5.850$$

For others samples the same calculation for the concentration and the percentage inhibition of the sample were done.

e.g. Calculation the sample concentration at  $IC_{50}$  of SPT1 2 weeks without soybean milk



**Figure B1.2** Show as the linear equation from standard calibration curve for was % inhibition of SPT1 2 weeks without soybean milk

$$Y = 503.56X - 12.683$$

The calculation of  $IC_{50}$  of the samples is shown below.

$$Y = 50 \% \text{ inhibition } (IC_{50})$$

$$X = \text{concentration of sample}$$

e.g. SPT1 2 weeks without soybean milk ;

$$X = \frac{Y}{503.56} + 12.683$$

$$= \frac{50}{503.56}$$

$$= \frac{50 + 12.683}{503.56}$$

$$= 0.124479 \text{ mg/ml}$$

For others samples the same calculation for the  $IC_{50}$  were done, and show in Table ..

**Table B1.3** Shown IC<sub>50</sub> (μg/ml for BHT the others mg/ml) calculated from graph

Fermented rice	IC <sub>50</sub>	IC <sub>50</sub>	Average ± SD
BHT	0.53891	0.546341	0.542625±0.005254
Commercial red yeast rice	ND	ND	ND
Mali105	ND	ND	ND
Mali105 <sup>a</sup> 2 weeks	0.237921	0.223769	0.230845±0.010007
Mali105 <sup>a</sup> 3 weeks	0.098418	0.103308	0.100863±0.003457
Mali105 <sup>b</sup> 2 weeks	0.241522	0.23797	0.239746±0.010007
Mali105 <sup>b</sup> 3 weeks	0.173839	0.185859	0.179849±0.010007
Km	0.222946	0.224433	0.223689±0.001051
Kam <sup>a</sup> 2 weeks	0.170316	0.160281	0.165298±0.007096
Kam <sup>a</sup> 3 weeks	0.189616	0.195016	0.192316±0.003819
Kam <sup>b</sup> 2 weeks	0.102395	0.104036	0.103215±0.00116
Kam <sup>b</sup> 3 weeks	0.105913	0.11311	0.109512±0.00509
RD6	ND	ND	ND
RD6 <sup>a</sup> 2 weeks	0.172255	0.17783	0.175043±0.003942
RD6 <sup>a</sup> 3 weeks	0.119489	0.125204	0.122347±0.004041
RD6 <sup>b</sup> 2 weeks	0.097807	0.087247	0.092527±0.007467
RD6 <sup>b</sup> 3 weeks	0.085953	0.086342	0.086148±0.000275

<b>Fermented rice</b>	<b>IC<sub>50</sub></b>	<b>IC<sub>50</sub></b>	<b>Average <math>\pm</math> SD</b>
SPT1	ND	ND	ND
SPT1 <sup>a</sup> 2 weeks	0.12448	0.123979	0.12423 $\pm$ 0.000354
SPT1 <sup>a</sup> 3 weeks	0.110517	0.109384	0.109951 $\pm$ 0.000802
SPT1 <sup>b</sup> 2 weeks	0.066532	0.102839	0.084686 $\pm$ 0.025674
SPT1 <sup>b</sup> 3 weeks	0.089321	0.085126	0.087224 $\pm$ 0.002967

ND = not detectable

<sup>a</sup> Fermented without soybean milk, <sup>b</sup> Fermented with soybean milk

**B6. Measuring the content of citrinin content**

Fermented rice (red yeast rice) 2.5 g in 15 ml 70 % ethanol extract, Calculated citrinin contents from standard calibration curve ( shown in Figure B1.3 ) for HPLC technique.

**Sample quantification**

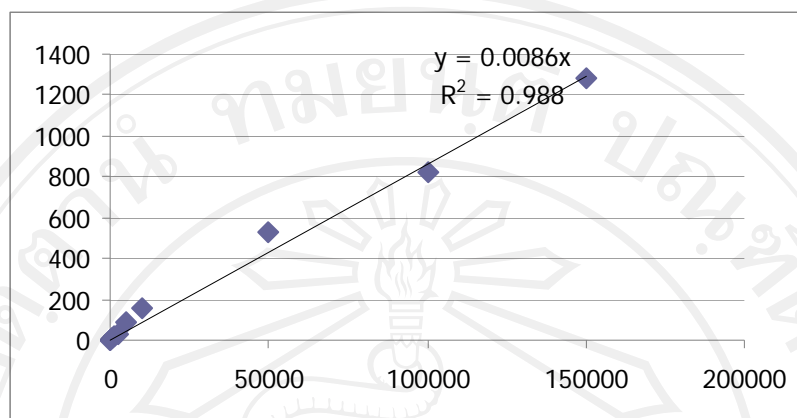
The standard calibration curve of standard citrinin was prepared using 150, 313, 625, 1250, 2500, 5000, 10000, 50000, 100,000 and 150,000 ppb. The solutions were filtered with 0.20  $\mu\text{m}$  nylon membrane before use. All of the standard citrinin was injected in triplicates time in the volume of 10  $\mu\text{l}$ .

To determine the citrinin content in the samples, the content of citrinin in all of the samples except red yeast rice were quantified using HPLC technique. The samples were injected according to the volume of the area in the standard curve.

**Table B1.4** Peak area content of standard citrinin for different concentration

Standard citrinin concentration (ppb)	Peak area 1	Peak area 2	Average
150,000	1299.90	1258.90	1279.40
100,000	660.40	976.60	818.50
500,000	590.00	476.20	533.10
10,000	145.30	176.70	161.00
5,000	84.90	83.50	84.20
1250	18.77	18.02	18.40
625	6.34	5.78	6.068
313	2.85	2.78	2.81
150	Not detectable	Not detectable	Not detectable

The peak area was used to plot the standard calibration curve versus the concentration



**Figure B1.3** The standard calibration curve of citrinin

The linear equation from standard calibration curve for citrinin content was

$$Y = 0.0086X$$

Y = peak area average of red yeast rice ( from chromatogram)

X = citrinin content (ppb)

Calculated citrinin content from equation from standard calibration curve;

$$X = \frac{Y}{0.0086}$$

e.g. citrinin content of Comryr; =  $\frac{21.71}{0.0086} = 2524.42$  ppb

citrinin content of Comryr 2524.42 ppb =  $\frac{2524.42}{1000}$  ppm

1000

The extract of Comryr 1000 ml citrinin content  $\frac{2524.42}{1000}$  mg

1000

The extract of Comryr 0.5 ml citrinin content  $\frac{0.5 \times 2524.42}{1000} \text{ mg}$

1000 x 1000

The extract of Comryr 5 ml citrinin content  $\frac{0.5 \times 2524.42 \times 5}{1000} \text{ mg}$

1000 x 1000

The extract of Comryr 15 ml from Comryr 2.5 g

Comryr 2.5 g citrinin content  $\frac{0.5 \times 2524.42 \times 5}{1000} \text{ mg}$

1000 x 1000

Comryr 1 g citrinin content  $\frac{0.5 \times 2524.42 \times 5}{2.5 \times 1000} \text{ mg}$

2.5 x 1000 x 1000

Citrinin content of Comryr = 0.0025 mg/g

Table B1.5 and 1.6 show the content of citrinin calculated from the standard calibration curve.

**Table B1.5** Citrinin content in 2, 3 weeks old red yeast rice without soybean milk

<b>Fermented rice</b>	<b>Peak area1</b>	<b>Peak area2</b>	<b>Average</b>	<b>Citrinin content (ppb)</b>	<b>Citrinin content (mg/g)</b>
Comryr	32.72	10.69	21.71	2524.42	0.0025
Mali105 2 weeks	5.23	-	5.23	608.14	0.0006
Mali105 3 weeks	9.84	-	9.84	1136.25	0.001
Kam 2 weeks	79.86	76.393	78.13	9084.88	0.009
Kam 3 weeks	66.69	98.795	82.74	9620.93	0.006
RD6 2 weeks	126.98	103.76	115.37	13415.12	0.013
RD6 3 weeks	37.14	98.097	67.62	7862.62	0.008
SPT1 2 weeks	342.72	333.47	338.09	39312.20	0.039
SPT1 3 weeks	126.43	119.88	123.16	14221.71	0.014

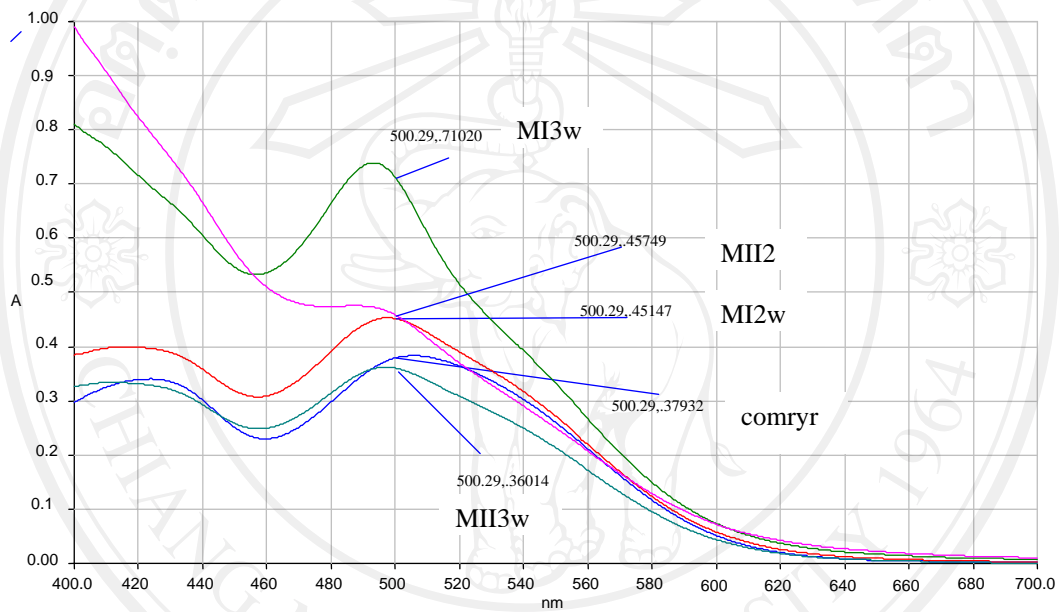
**Table B1.6** Citrinin content in 2, 3 weeks old red yeast rice with soybean milk

<b>Fermented rice</b>	<b>Peak area1</b>	<b>Peak area2</b>	<b>Average</b>	<b>Citrinin content (ppb)</b>	<b>Citrinin content (mg/g)</b>
Mali105 2 weeks	6.00	-	6.00	697.67	0.0007
Mali105 3 weeks	3.49	-	3.49	405.81	0.0004
Kam 2 weeks	66.70	43.895	55.30	6430.23	0.006
Kam 3 weeks	56.63	41.086	48.86	5681.39	0.005
RD6 2 weeks	95.52	82.586	89.05	10282.91	0.010
RD6 3 weeks	18.31	56.897	37.60	4372.09	0.004
SPT1 2 weeks	425.86	431.58	428.72	49851.16	0.049
SPT1 3 weeks	250.18	252.40	251.29	29220.81	0.029

## APPENDIX C

## Graph

## C1. Graph of spectrum red pigment



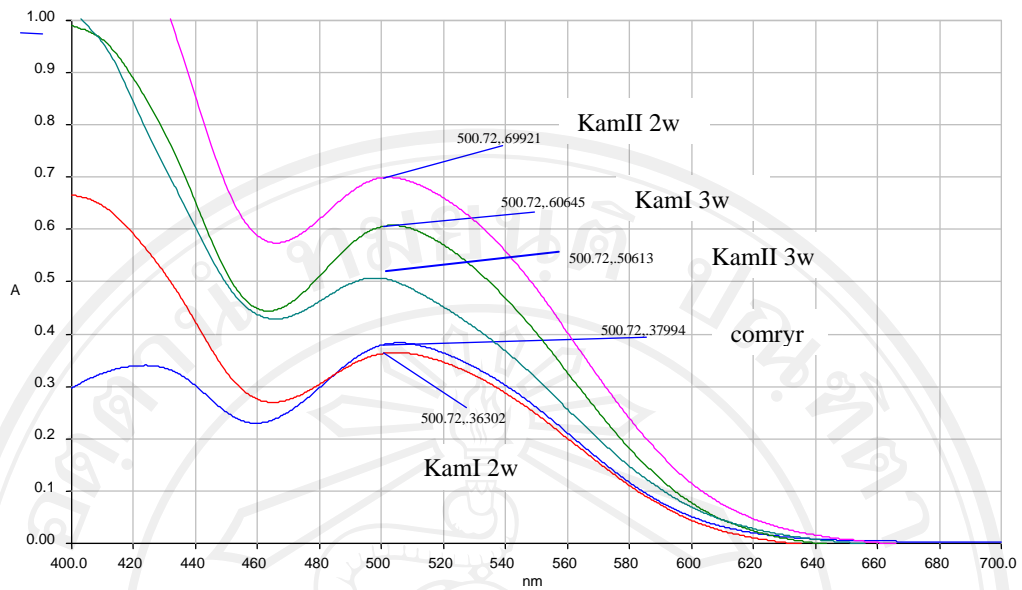
Comryr= Commercial Chinese red yeast rice

MI 2w = Mali105 2weeks without soybean milk

MI 3w = Mali105 3weeks without soybean milk

MII2w = Mali105 2weeks with soybean milk

MII3w = Mali105 3weeks with soybean milk



Comryr = Commercial Chinese red yeast rice

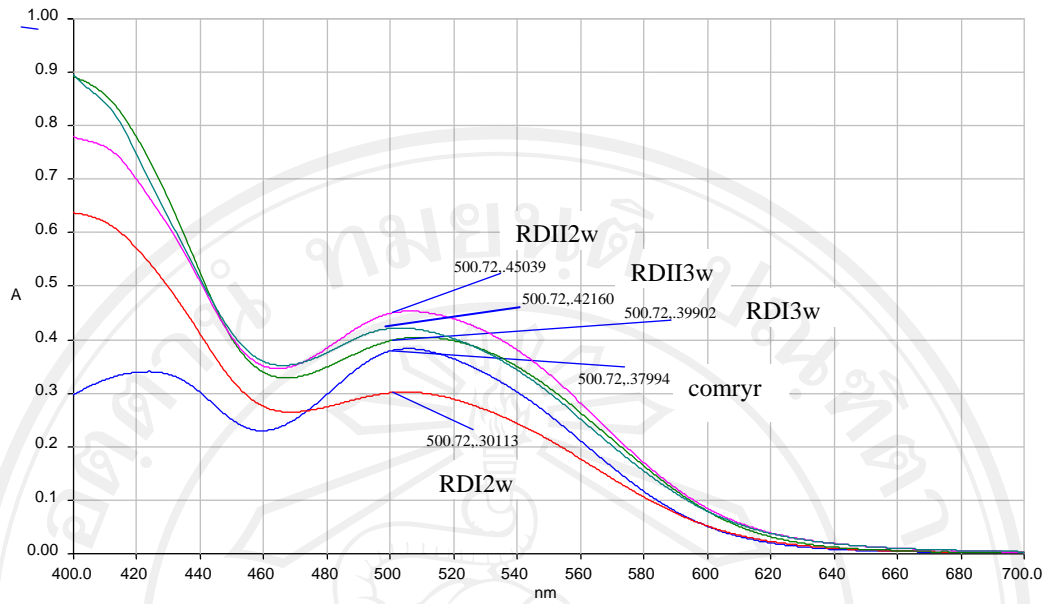
KamI 2w = Kam 2weeks without soybean milk

KamI 3w = Kam 3weeks without soybean milk

KamII2w = Kam 2weeks with soybean milk

KamII3w = Kam 3weeks with soybean milk

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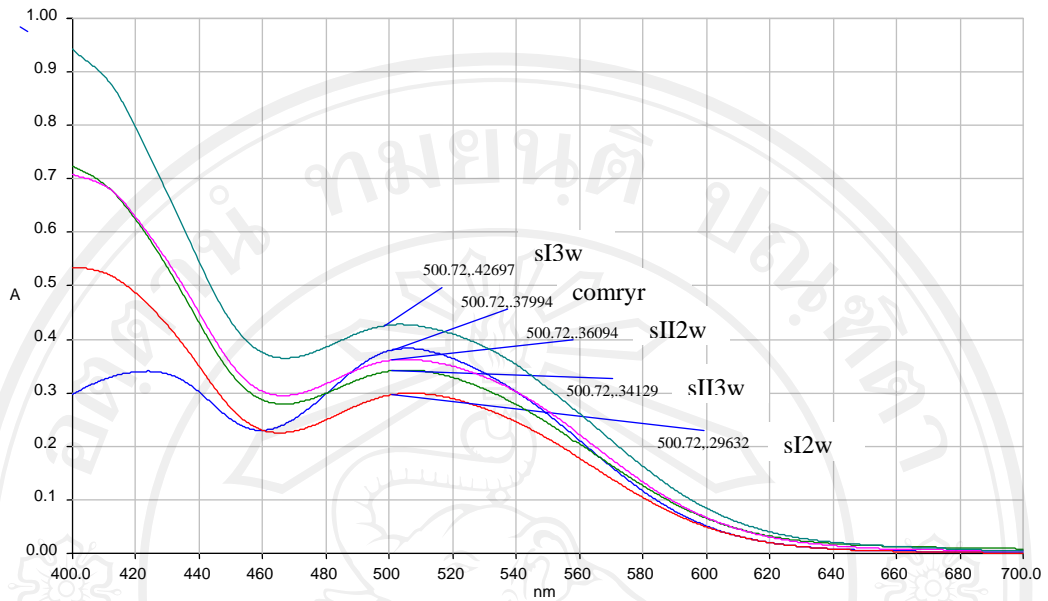
Comryr = Commercial Chinese red yeast rice

RDI2w = RD6 2 weeks without soybean milk

RDI3w = RD6 3 weeks without soybean milk

RDII2w = RD6 2 weeks with soybean milk

RDII3w = RD6 3 weeks with soybean milk



Comryr = Commercial Chinese red yeast rice

sI 2w = SPT1 2weeks without soybean milk

sI 3w = SPT1 3weeks without soybean milk

sII2w = SPT1 2weeks with soybean milk

sII3w = SPT1 3weeks with soybean milk

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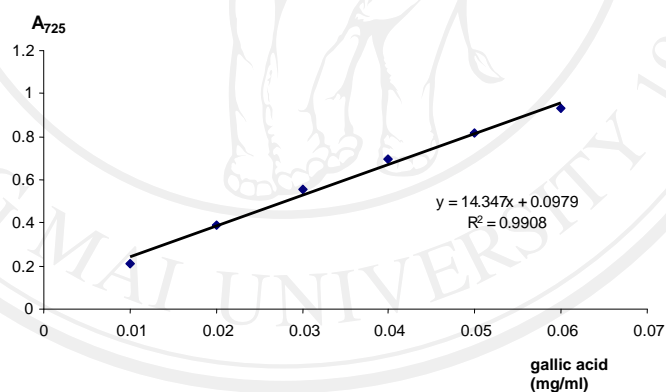
## C2. Antioxidant activities graph

### C2.1 Result of the antioxidant capacity

#### Graph of standard Gallic acid

Standard Gallic acid

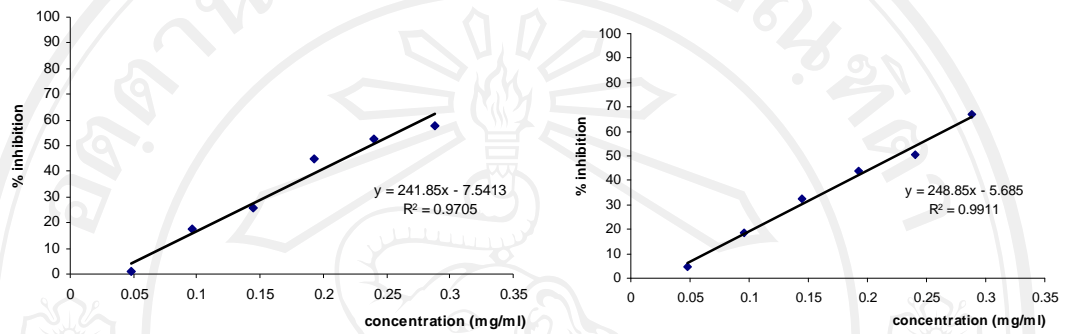
Tube	Gallic acid ( mg/ml)					
	0.01	0.02	0.03	0.04	0.05	0.06
A <sub>725</sub> (1)	0.207	0.387	0.562	0.696	0.826	0.929
A <sub>725</sub> (2)	0.211	0.396	0.551	0.696	0.810	0.930
Average	0.209	0.392	0.556	0.696	0.818	0.930



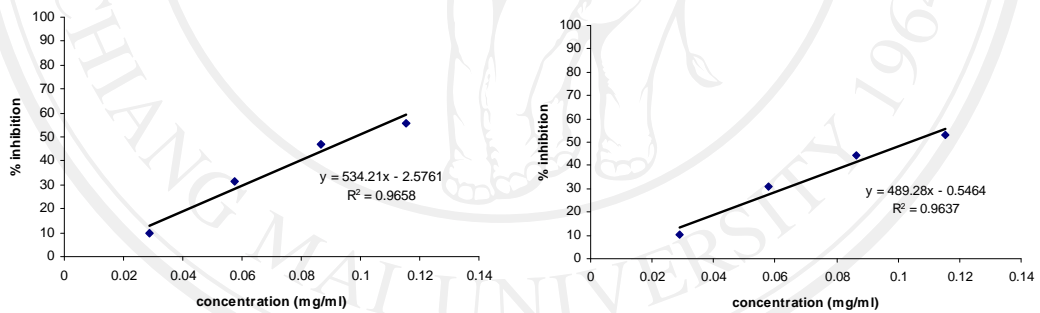
**C2.2 Result of the  $\beta$ -carotene bleaching activity**

**Graph of concentration and % inhibition**

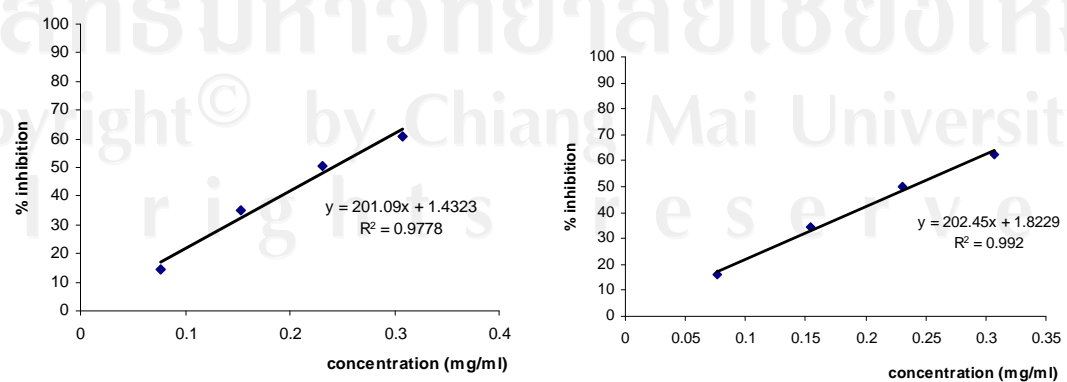
Mali105 2 weeks without soybean milk



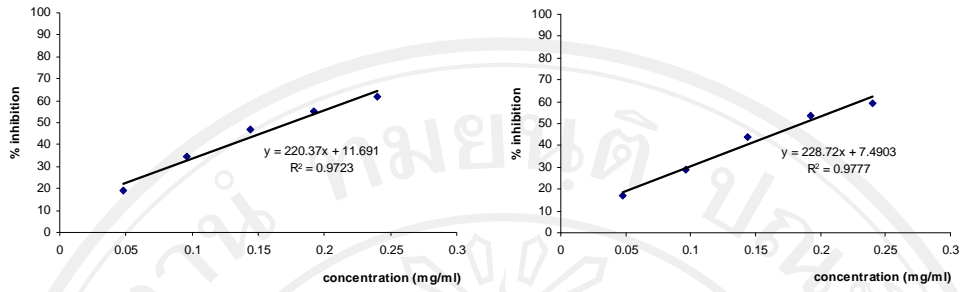
Mali105 3 weeks without soybean milk



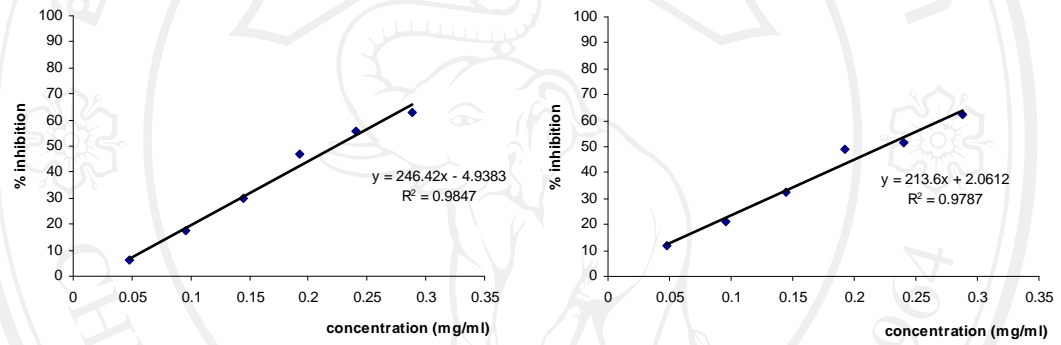
Mali105 2 weeks with soybean milk



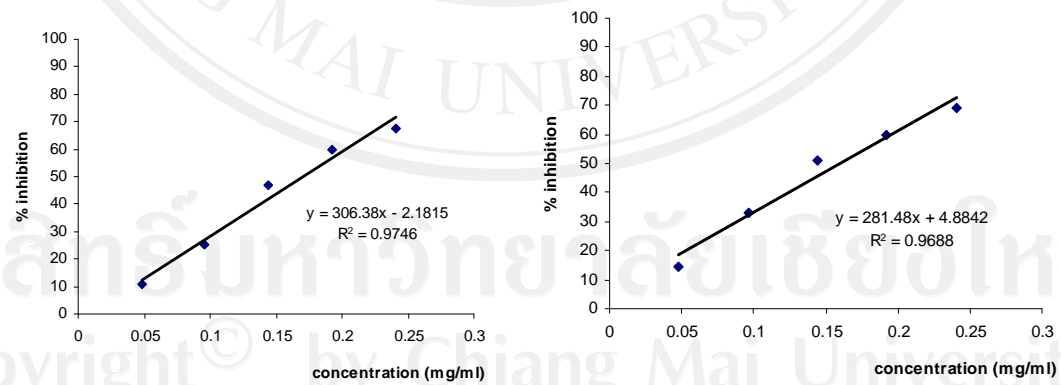
Mali105 3 weeks with soybean milk



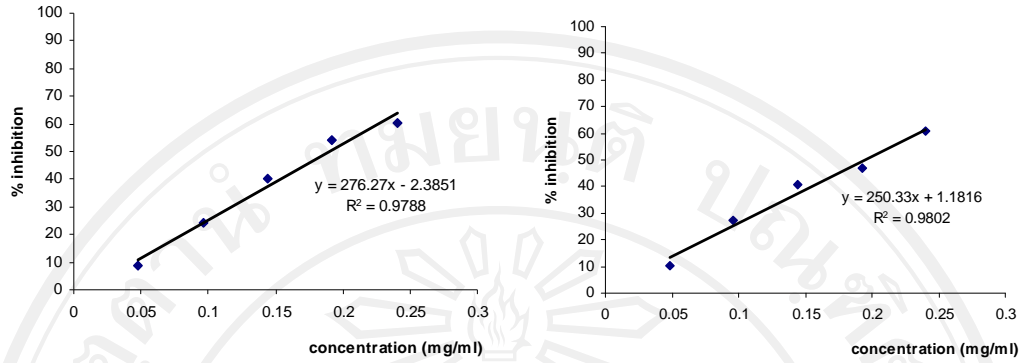
Kam ( not fermented)



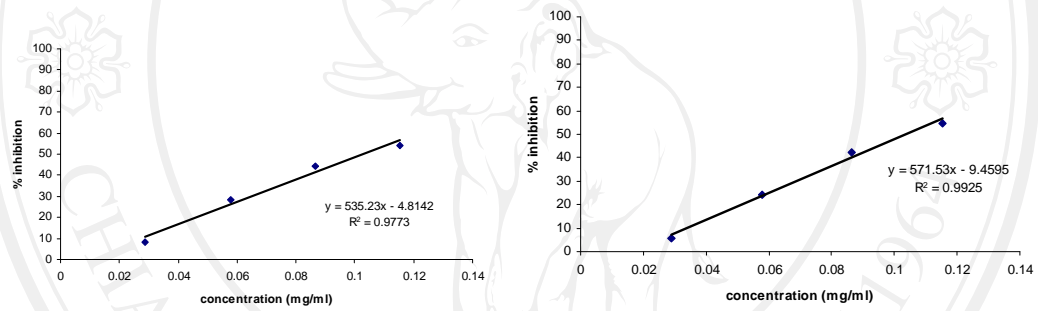
Kam 2 weeks without soybean milk



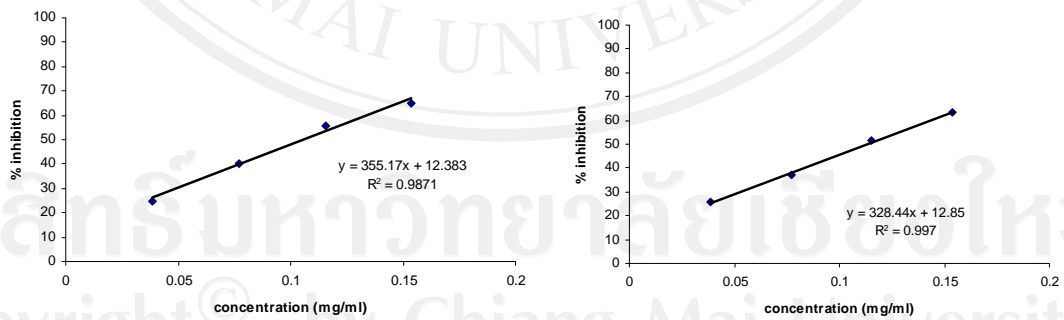
Kam 3 weeks without soybean milk



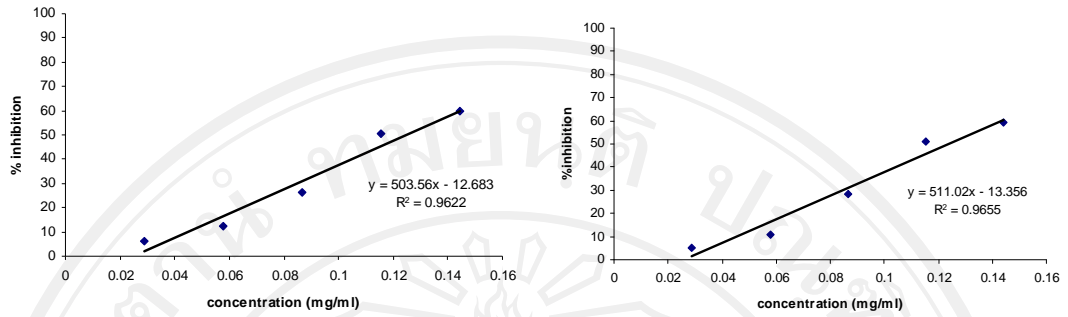
Kam 2 weeks with soybean milk



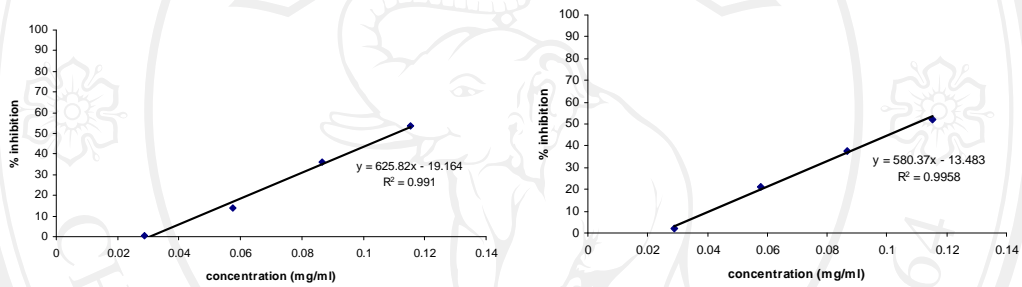
Kam 3 weeks with soybean milk



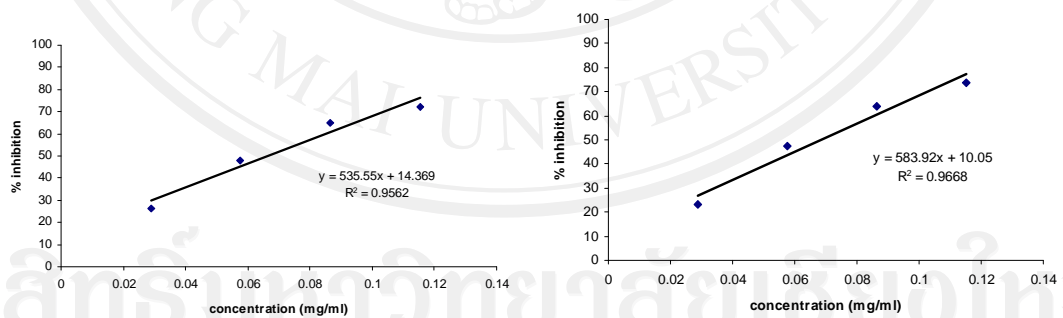
SPT1 2 weeks without soybean milk



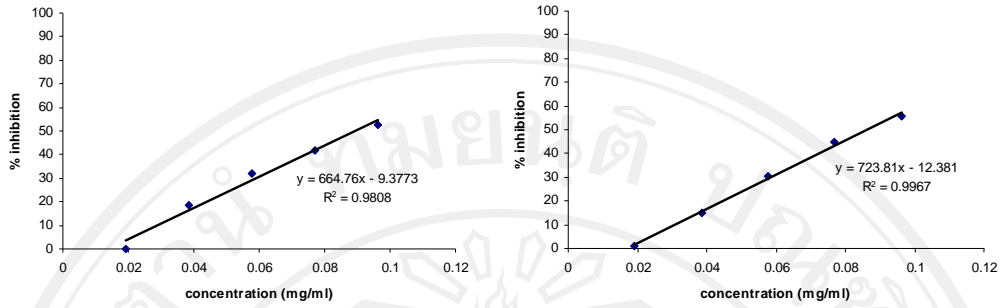
SPT1 3 weeks without soybean milk



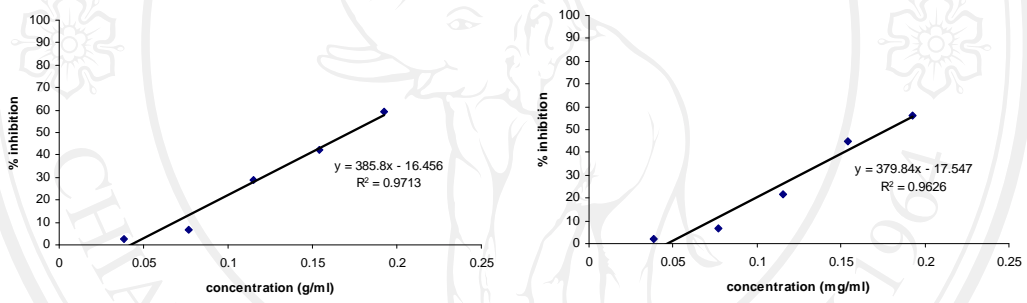
SPT1 2 weeks with soybean milk



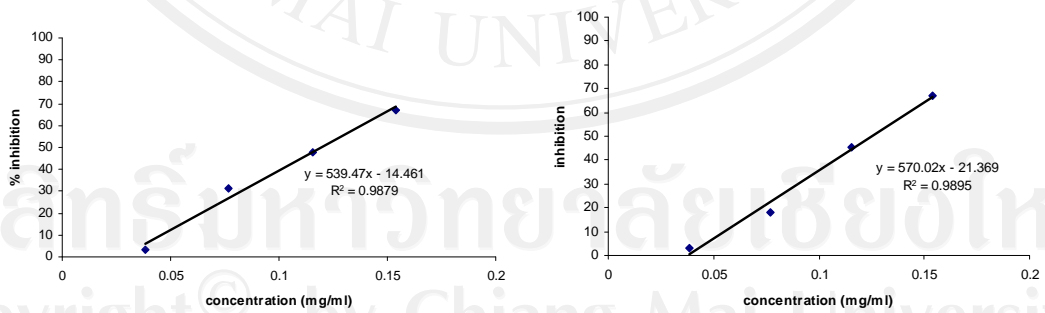
SPT1 3 weeks with soybean milk



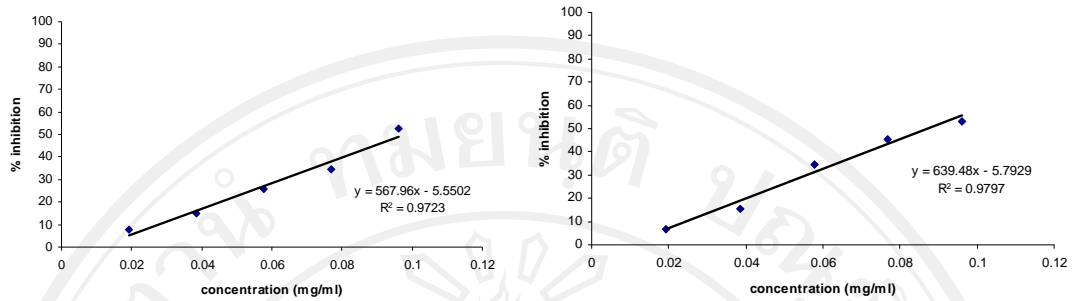
RD6 2 weeks without soybean milk



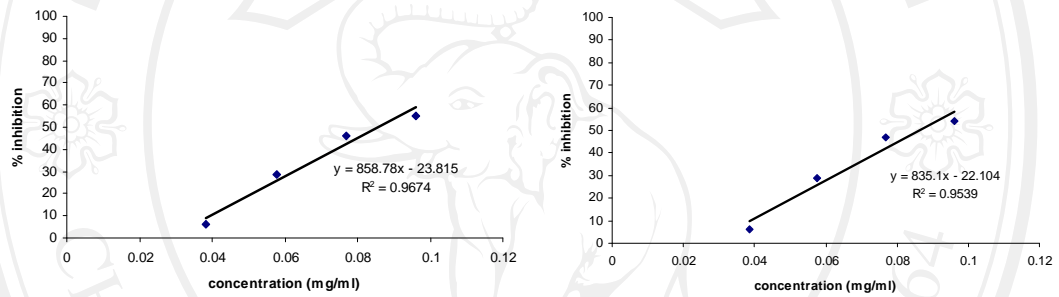
RD6 3 weeks withOut soybean milk



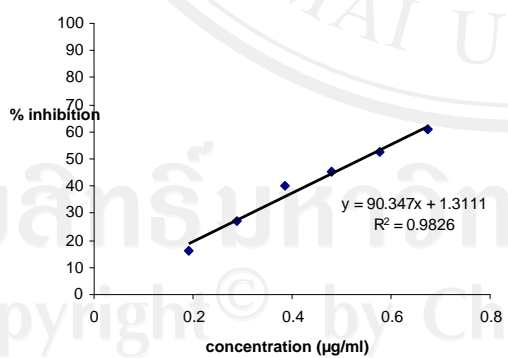
RD6 2 weeks with soybean milk



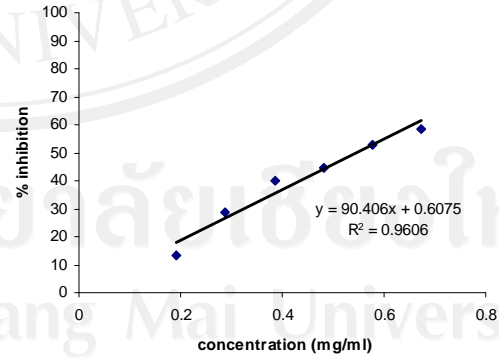
RD6 3 weeks with soybean milk



Standard BHT 1



Standard BHT 2



RD6, SPT1 , Mali105, Commercial Chinese red yeast rice = not detected

APPENDIX D

Chromatograms

D. Chromatograms of HPLC

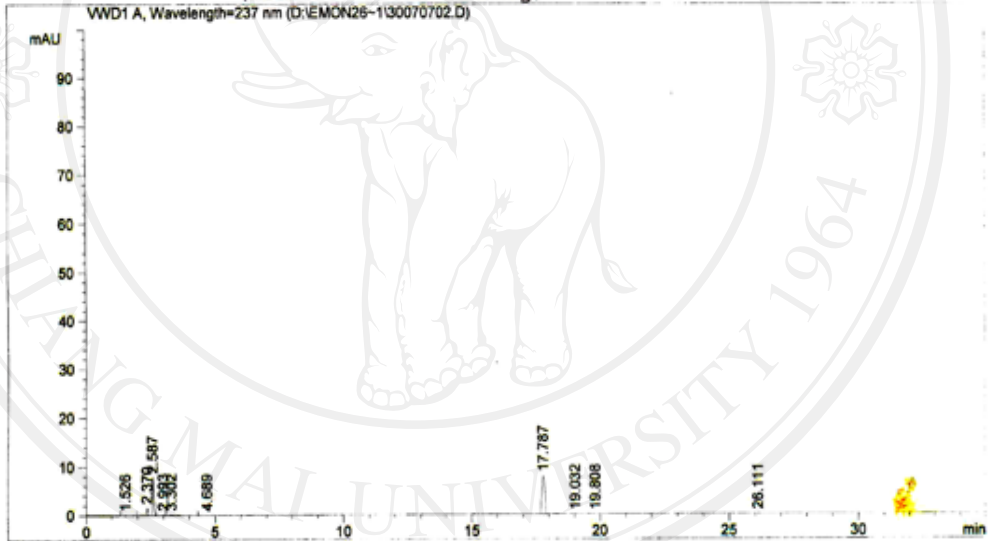
D1. Chromatograms of standard monacolin K

Data File D:\EMON26~1\30070702.D

Sample Name: std. Mevi 24 ju

```

-----
Injection Date : 7/30/2007 12:59:20 PM      Seq. Line : 2
Sample Name    : std. Mevi 24 ju            Vial : 2
Acq. Operator  : nont                       Inj  : 1
Acq. Method    : C:\HPCHEM\1\METHODS\MONACOLI.M
Last changed   : 7/30/2007 12:17:14 PM by nont
Analysis Method : C:\HPCHEM\1\METHODS\TEST13.M
Last changed   : 7/31/2007 3:00:20 PM
                (modified after loading)
    
```



Area Percent Report

```

-----
Sorted By      : Signal
Multiplier     : 1.0000
Dilution       : 1.0000
    
```

Signal 1: WVD1 A, Wavelength=237 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU	Height [mAU]	Area %
1	1.526	BV	0.5887	54.16566	1.13514	6.44991
2	2.370	VP	0.2335	93.80754	5.13504	11.17037
3	2.587	VV	0.1030	79.53742	11.64610	9.47112
4	2.993	VV	0.2788	77.87416	3.55538	9.27306
5	3.302	VV	0.8007	213.24356	3.31175	25.39252
6	4.689	VB	0.7410	126.80441	2.07815	15.09956
7	17.787	PB	0.1703	126.45582	11.65081	15.05805
8	19.032	PV	0.2355	9.26629	5.72366e-1	1.10341
9	19.808	VB	0.2333	4.09911	2.56188e-1	0.48811
10	26.111	BP	1.0577	54.53501	6.34767e-1	6.49389

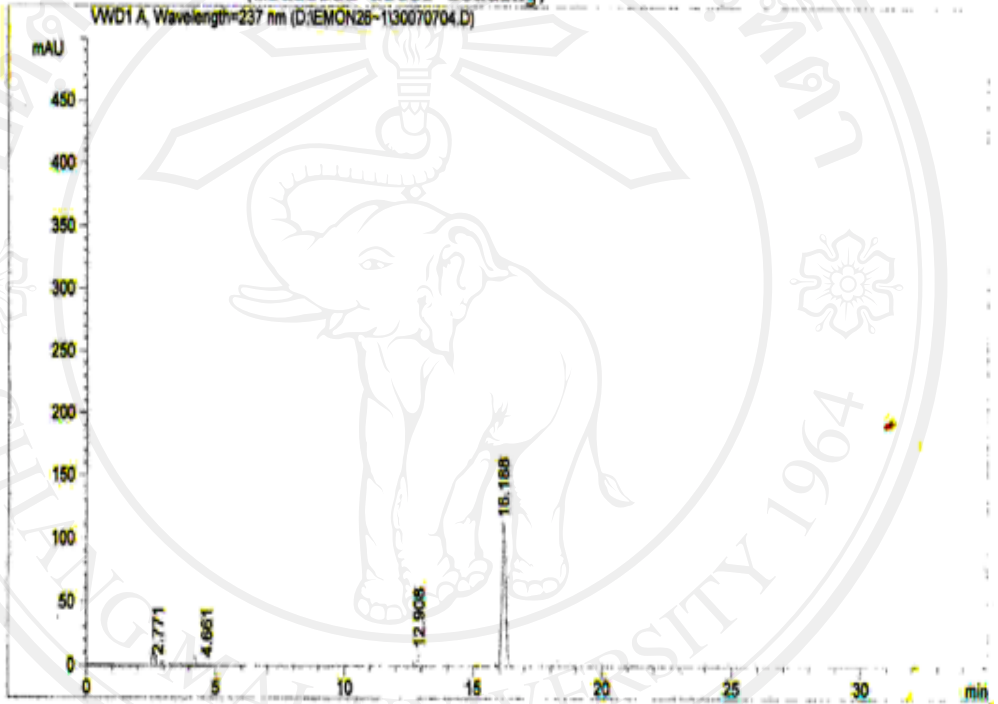
D2. Chromatograms of standard compactin

Data File D:\EMON26\1\30070704.D

Sample Name: std. Com new

```

=====
Injection Date : 7/30/2007 2:12:40 PM      Seq. Line : 4
Sample Name    : std. Com new              Vial      : 4
Acq. Operator  : nont                      Inj       : 1
Acq. Method    : C:\HPCHEM\1\METHODS\MONACOLI.M
Last changed   : 7/30/2007 12:17:14 PM by nont
Analysis Method : C:\HPCHEM\1\METHODS\TEST13.M
Last changed   : 7/31/2007 3:14:40 PM
                  (modified after loading)
=====
    
```



Area Percent Report

```

=====
Sorted By      : Signal
Multiplier    : 1.0000
Dilution      : 1.0000
    
```

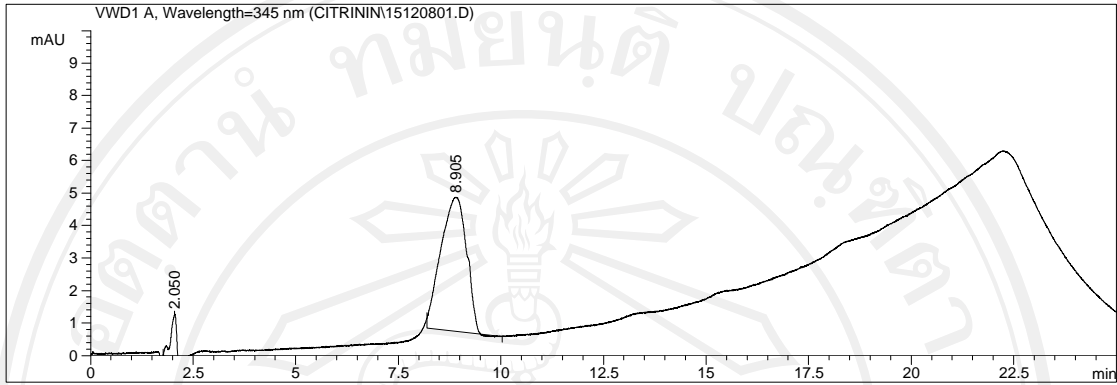
Signal 1: WVD1 A, Wavelength=237 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU*s	Height [mAU]	Area %
1	2.771	VV	0.1475	137.85165	12.37047	9.24711
2	4.661	DP	0.1319	20.30483	2.30415	1.36205

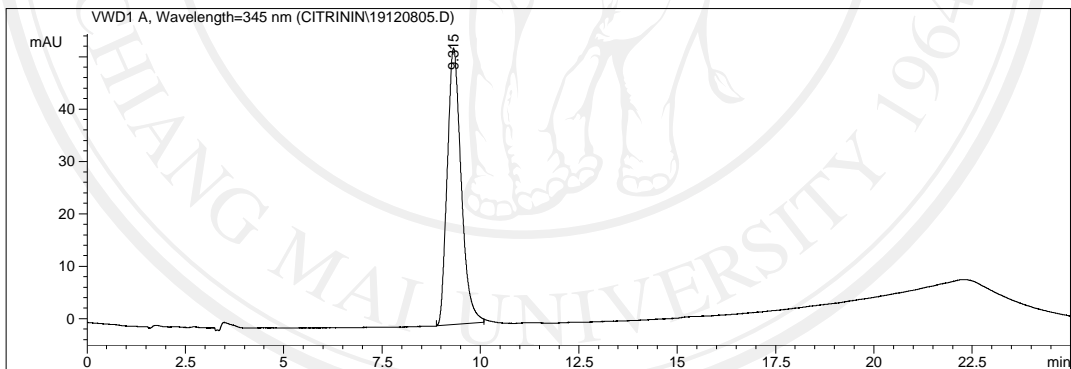
ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
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**D3. Chromatograms of chloroform and standard citrinin**

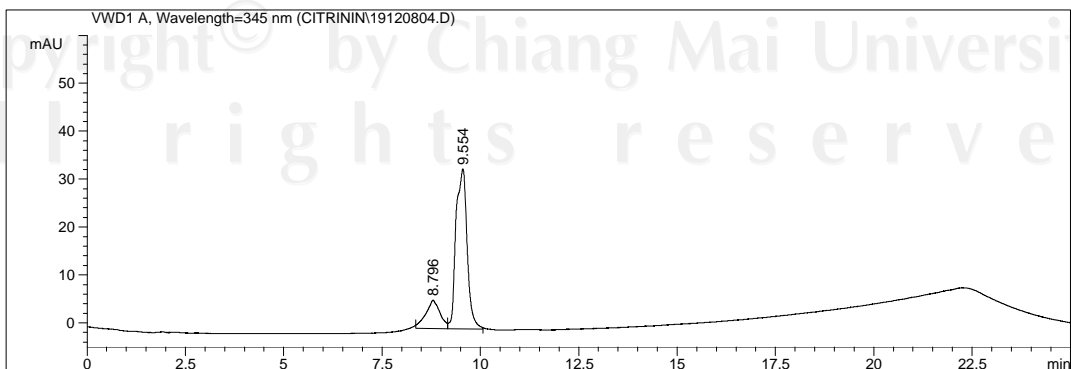
chloroform



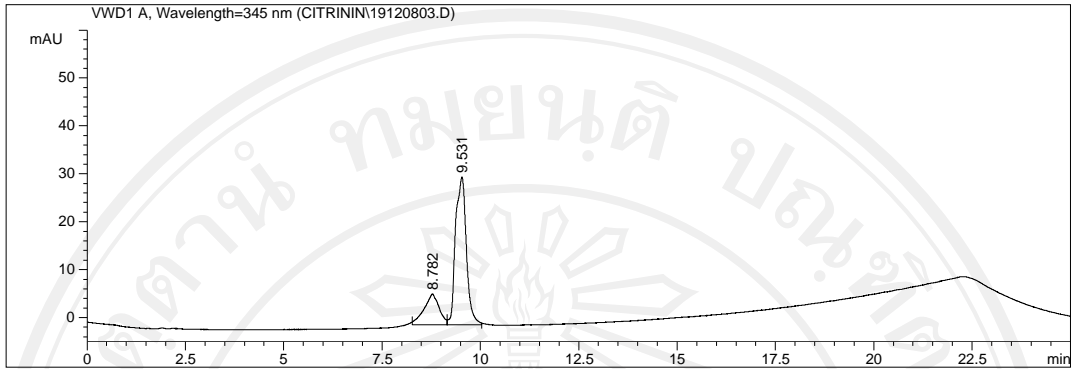
citrinin concentrated 150000 ppb



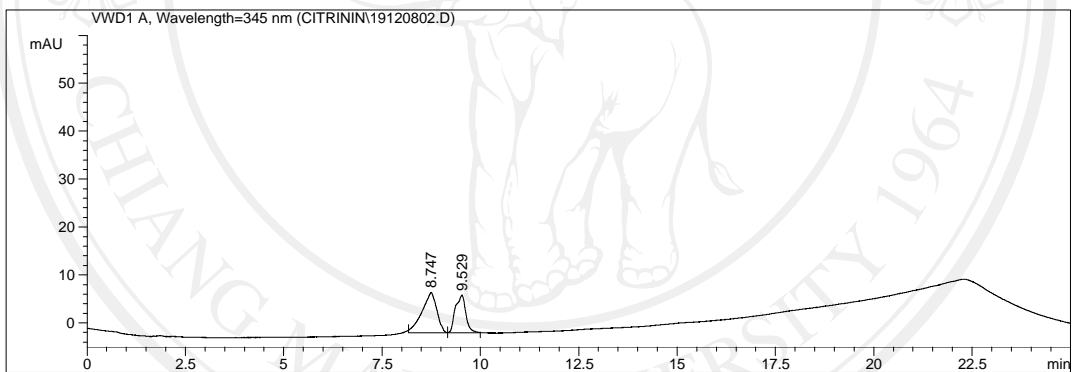
citrinin concentrated 100000 ppb



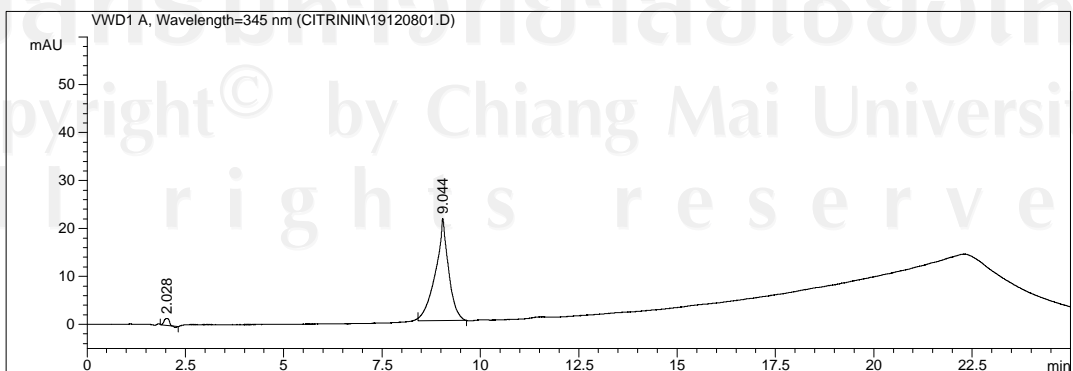
citrinin concentrated 50000 ppb



citrinin concentrated 10000 ppb

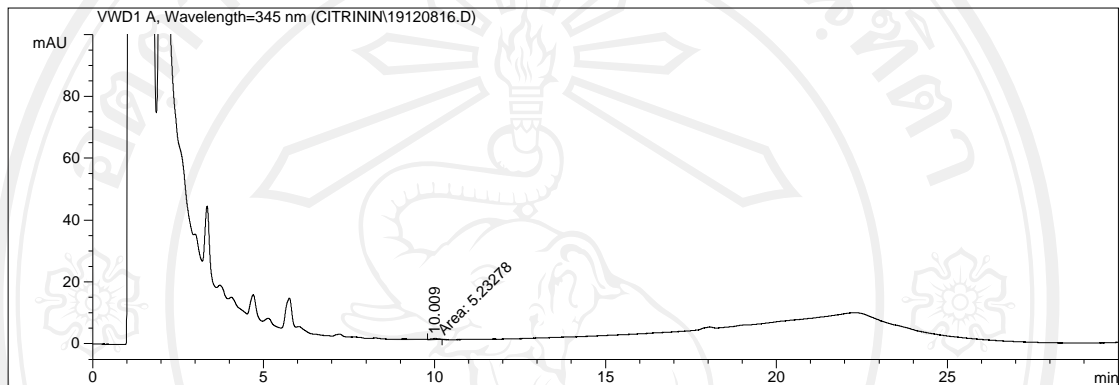


citrinin concentrated 300 ppb

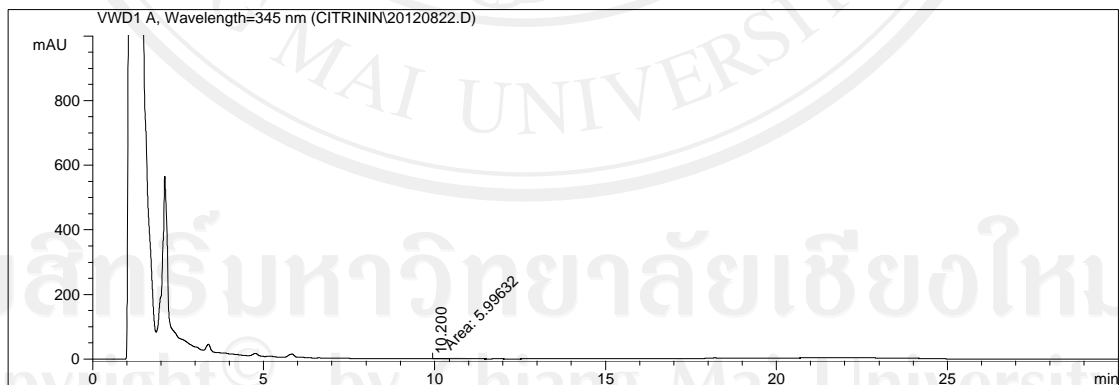


**D4.** Chromatographic chemical profiling of citrinin in fermented red yeast rice from non-glutinous rice and glutinous rice for 2 weeks

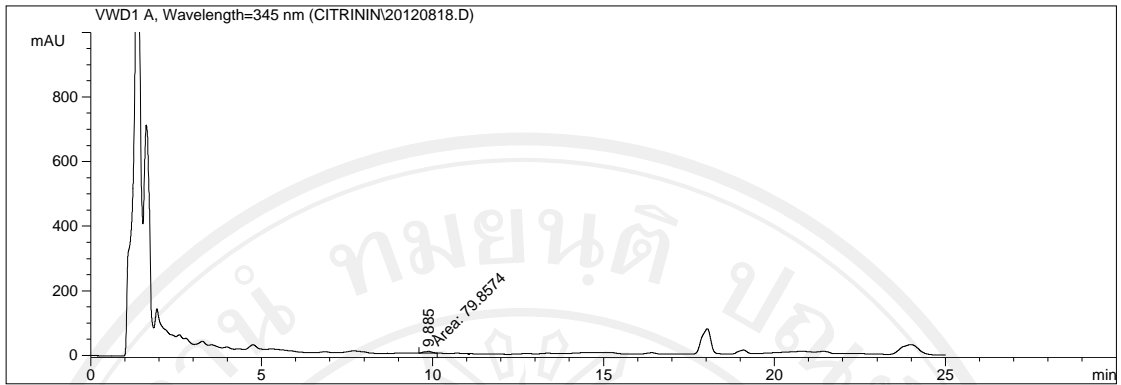
*Oryza sativa* L. cv. Mali105 2 weeks without soybean milk



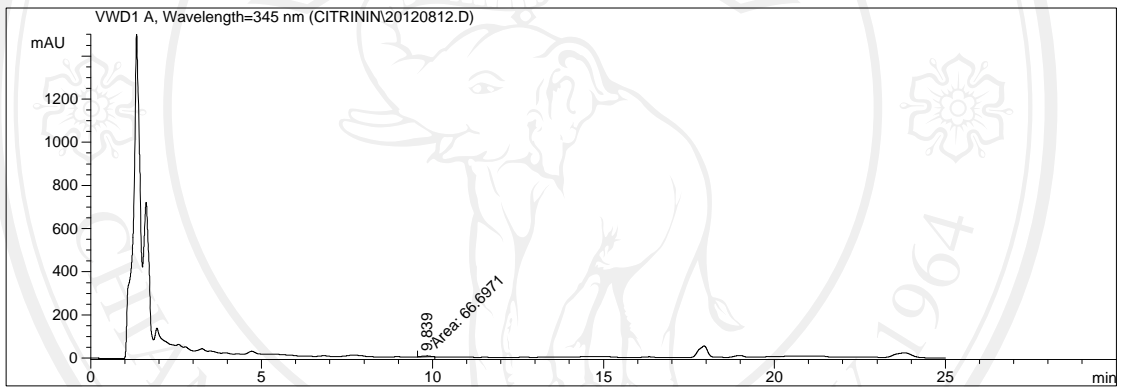
*Oryza sativa* L. cv. Mali105 2 weeks with soybean milk



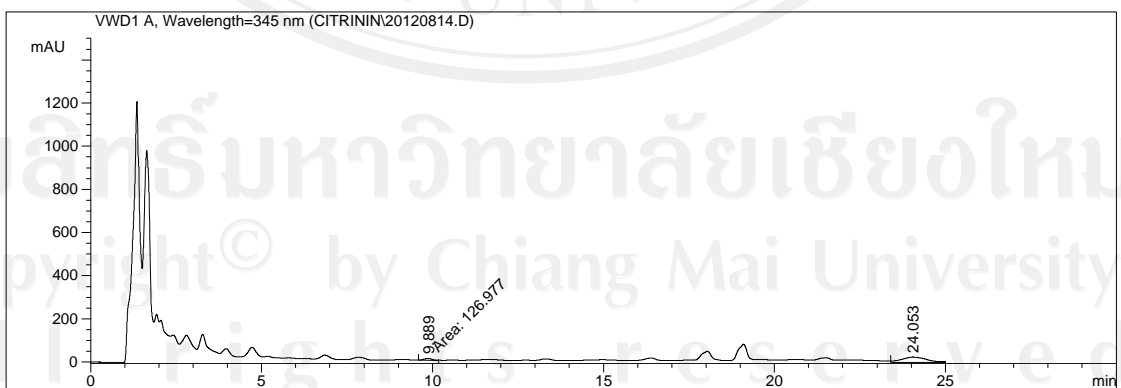
*Oryza sativa* L. cv. Kam 2 weeks without soybean milk



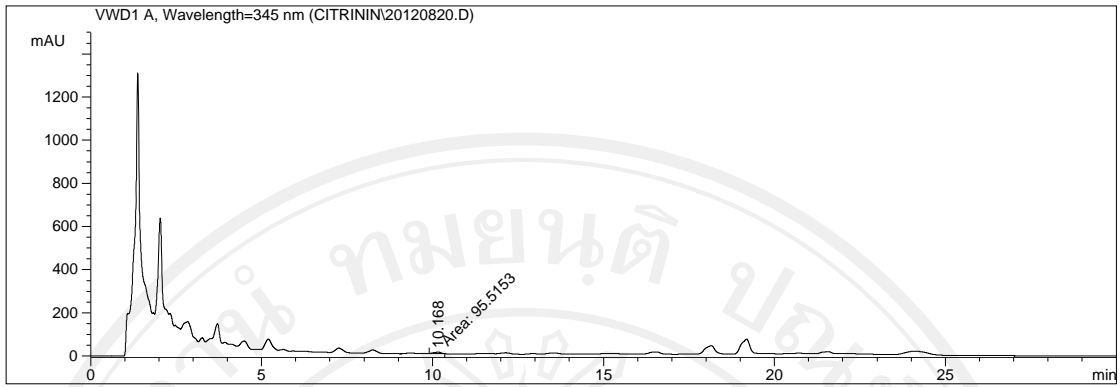
*Oryza sativa* L. cv. Kam 2 weeks with soybean milk



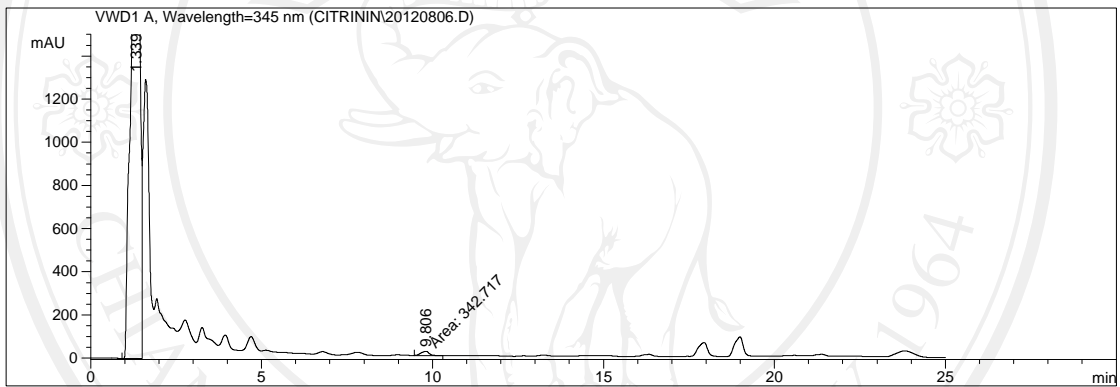
*Oryza sativa* L. cv. RD6 2 weeks without soybean milk



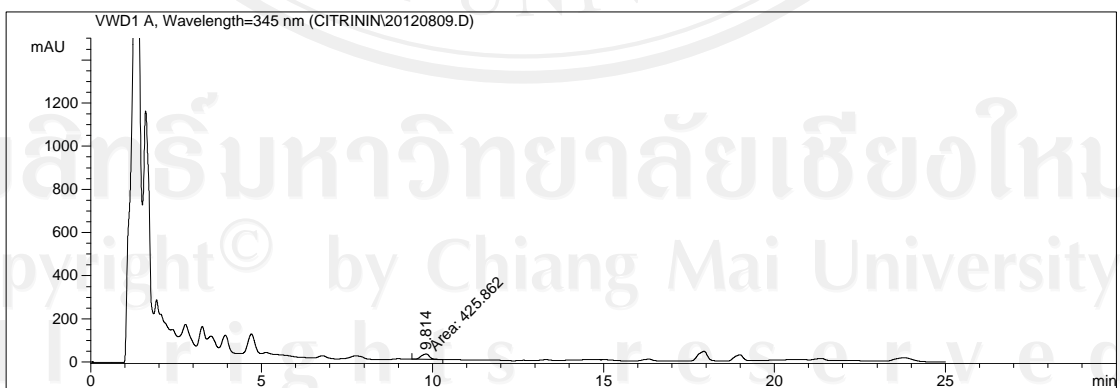
*Oryza sativa* L. cv. RD6 2 weeks with soybean milk



*Oryza sativa* L. cv. SPT1 2 weeks without soybean milk



*Oryza sativa* L. cv. SPT1 2 weeks with soybean milk



**CURRICULUM VITAE**

**Name** Mrs. Em-on Chairote

**Date of Birth** September 13, 1960

**Place of Birth** Nakhon Sawan, Thailand

**Home Address** 145/4 Tambol Rimthai, Amphoe Mae Rim,  
Chiang Mai, 50180 Thailand

**E-mail Address** [em\\_onchairote@hotmail.com](mailto:em_onchairote@hotmail.com)

**Education Background** B.A. (Education Science), Chiang Mai University  
1982.  
M.S. (Teaching Chemistry), Chiang Mai University  
1986.

**Position** Assistant Professor in Chemistry  
Level 8

**Institution** Faculty of Science and Agricultural Technology,  
Chiang Mai Campus,  
Rajamangala University of Technology Lanna  
(RMUTL)

### Publications

1. Chairote, E., Chairote, G., Wongpornchai, S. and Lumyong, S. (2007). Preparation of Red Yeast Rice Using Various Thai Glutinous Rice and *Monascus purpureus* Isolated from Commercial Chinese Red Rice Sample. KMITL Science and Technology Journal 7 (S1): 28-37.
2. Chairote, E., Chairote, G., Niamsup, H., and Lumyong, S. (2008). The Presence and the Content of Monacolins in Red Yeast Rice Prepared from Thai Glutinous Rice. World Journal Microbiol Biotechnology 24: 3039-3047.
3. Chairote, E., Chairote, G., and Lumyong, S. (2009). Red Yeast Rice Prepared from Thai Glutinous Rice and the Antioxidant Activities. Chiang Mai Journal of Science. 36(1): 42-49.
4. Chairote, E. (2004). Fundamental Chemistry for Industrial Technician. Rajamangala University of Technology Lanna. (in Thai)

### Presentation in international conference

- Chairote, E., Chairote, G., Wongpornchai, S., and Lumyong, S. (2007). Preparation of Red Yeast Rice Using Various Thai Glutinous Rice and *Monascus purpureus* Isolated from Commercial Chinese Red Rice Sample. The 5<sup>th</sup> International symposium on Biocontrol and Biotechnology, November 1-3, Nongkhai Campus, Khon Kaen University, Thailand.