Appendix B

Determination of protein by Lowry method (Lowry, 1951)

1. A sample 0.2 ml and add 1.0 ml of reagent C.
2. Mix and stand for 10 min.
3. Add 0.1 ml of Folin-ciocalteu reagent.
4. Mix and stand for 30 min.
5. Measure absorbance at 660 nm against a blank of 0.2 ml of distilleded water instead of sample processed through steps 1-4.
6. Bovine serum albumin solution was prepared to final concentration at 50, 100, 150, 200, 300 and 400 mg/ml for standard curve.

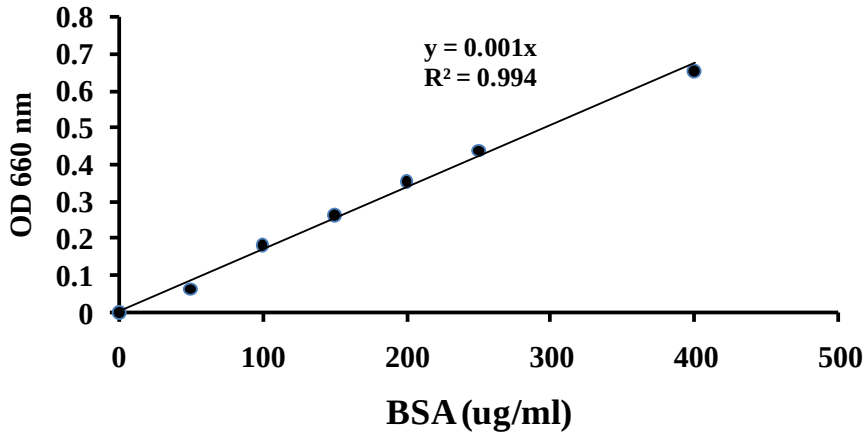

Figure B.1 Standard curve of bovine serium albumin (BSA) solution

Determination of reducing sugars by Somogyi-Nelson method (Nelson, 1944)

1. Take 0.6 ml sample and add 1 ml of Somogyi reagent.
2. Reaction mixture tube was boiled for 15 min.
3. Stand until cool, add 1 ml of Nelson reagent and mix well.
4. Add 2 ml of distilled water, mix and centrifuge at 10,000 rpm for 5 min.
5. Measure absorbance at 520 nm of supernatant against a blank of 0.6 ml of distilled water of instead sample processed through steps 1-4.

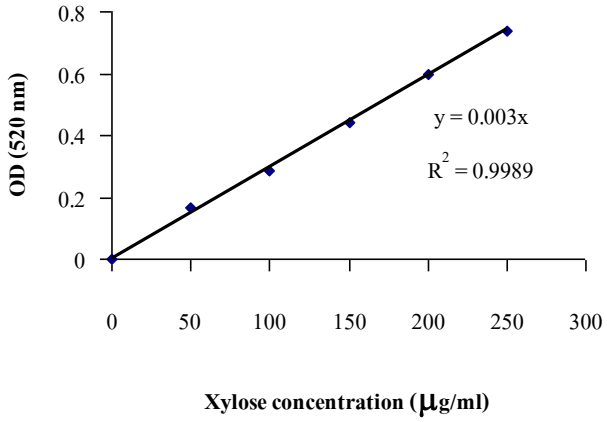

Figure B.2 Standard curve of xylose solution

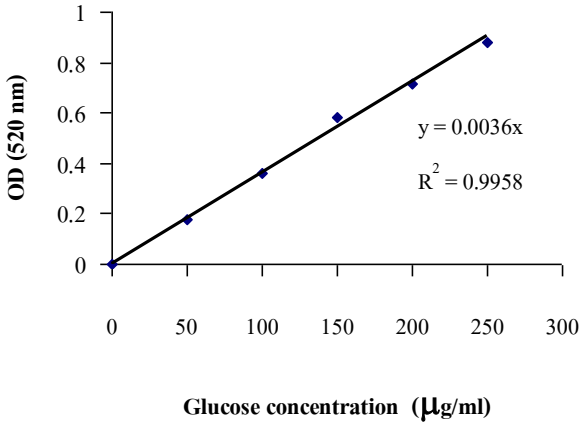

Figure B.3 Standard curve of glucose solution

Preparation of *p*-nitrophenol standard curve

1. The *p*-nitrophenol solution was prepared to final concentration at 5, 10, 15, 20, 25 and 50 μg/ml for standard curve.
2. Each concentration of *p*-nitrophenol solution (1.1 ml) was added into test tube and stopped reaction with 2 ml of 0.4 M Na_2CO_3.
3. Measured absorbance at 405 nm against a blank of 1.1 ml of distilleded water instead of sample processed through steps 1-2.

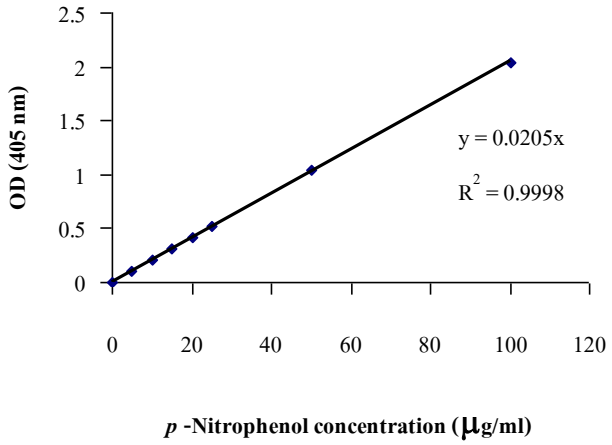

Figure B.4 Standard curve of *p*-nitrophenol solution

Electrophoresis method (Laemmli, 1970)

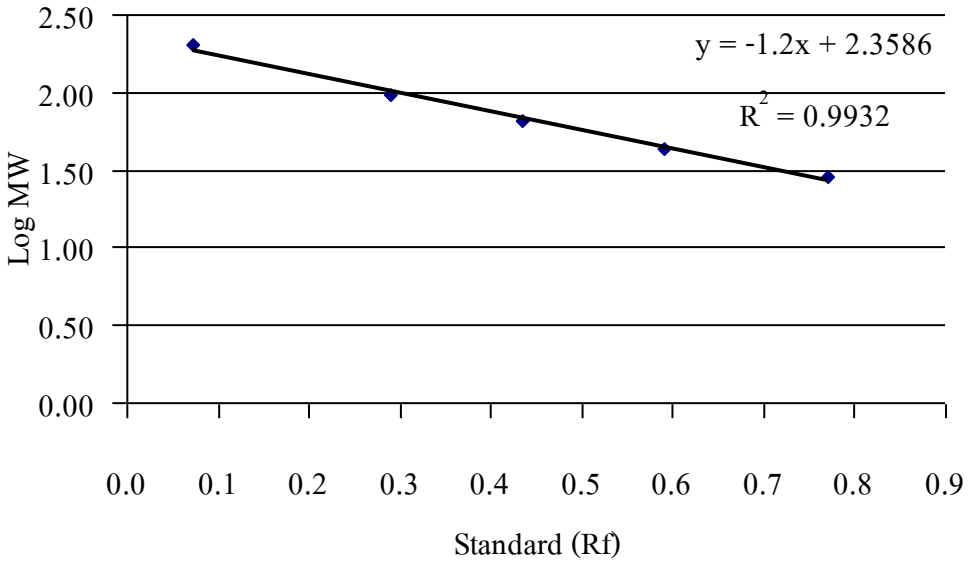

Figure B.5 Standard protein pattern of 10% SDS-PAGE

Zymogram (Ratanakhanokchai, 1999)

1-4. Prepare gel like SDS-PAGE method but add 0.2% substrate (soluble xylan or CMC) in separating gel.

5. Applied samples which want to know their purity and find theirs molecular. After boiled with sample buffer at 100 °C, 4 min and cooled then fill them in the gap above stacking gel while applied standard protein at the same time.

6-9 Do as SDS-PAGE method.

10. Wash SDS out of gel with 25% isopropanol 1-2 times.

11. Continue washing with sodium phosphate buffer 4 times, 30 min at 4 °C

12. Submerge gel in sodium phosphate buffer and incubated 50 °C, 1 hr.

13. Adsorb color by submerge gel in congo red, 15 min.

14. Wash excess color with 1.0 M NaCl.

15. Stop reaction by add acetic acid.

Sephacryl S-300 Gel filtration

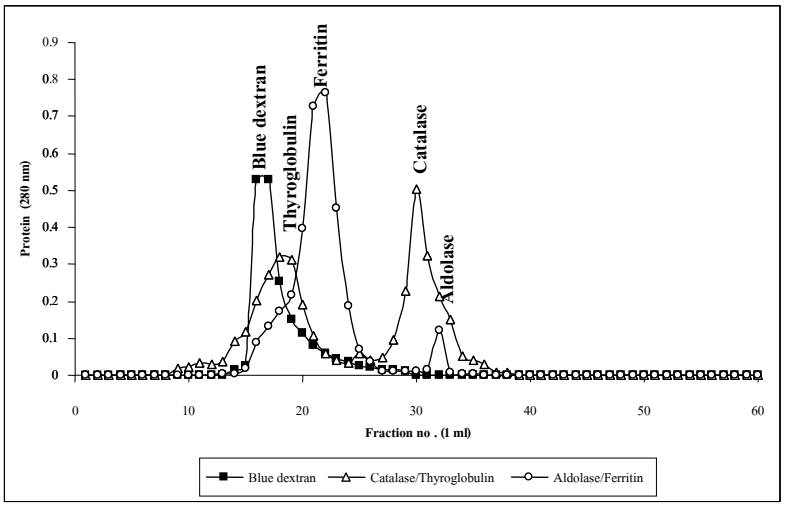

Figure B.6 Standard protein pattern of gel filtration (Sephacryl S-300); 0.67×50.0 cm^2, flow rate 0.3 ml/min., eluted with 50 mM phospate buffer pH 6.0

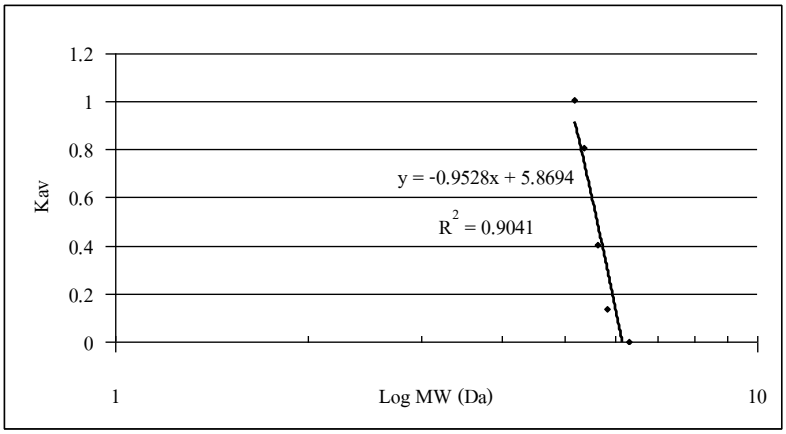

Figure B.7 Standard curve of standard protein for gel filtration (Sephacryl S-300); 0.67×50.0 cm^2, flow rate 0.3 ml/min., eluted with 50 mM phospate buffer pH 6.0