PROFILING OF PROTEIN INVOLVED IN TRUNKING AND NON-TRUNKING SAGO PALM

Nurhazlina Binti Hamdan

TP 248.27
P55
N974
2014
Bachelor of Science with Honours
(Resource Biotechnology)
2014
Acknowledgements

I would like to express my deepest appreciation to all those who provided me the possibility to complete this report. A special gratitude I give to our supervisor, Associate Professor Dr. Mohd Hasnain Md Hussain, for his patient guidance, enthusiastic encouragement, stimulating suggestions and useful critiques of this research work. And also for his contribution in helping me especially in coordinates my research and writing this report.

Furthermore I would also like to acknowledge with much appreciation to the Master student, Nikson Chong, Yan Wei Jie, Mohd. Izzuddin bin Alias and Mohd. Noorzafrudin bin Khairol, who teaches us how to use all required equipment and the necessary materials to complete this research. A special thanks goes to my team mate, Carmon Goh, Enna Rozzana binti Denis, Nur Ezzati binti Hamdin and Zulaikha binti Pol Ong, who help me and gave suggestion for this research. I have to appreciate the guidance given by other supervisor as well as the panels especially in our project presentation that has improved our presentation skills thanks to their comment and advices.

Additionally I would also like to thank my family especially my mother, who have supported me throughout the entire process, both by keeping me calm and helping me putting all the pieces together.
Declaration

UNIVERSITI MALAYSIA SARAWAK

Grade: ___________

Please tick (v)
Final Year Project Report
Masters
PhD

DECLARATION OF ORIGINAL WORK

This declaration is made on the 27 day of June 2014.

Student's Declaration:
I Nurhazlina Binti Hamdan, 32334, Faculty Resource Science and Technology (PLEASE INDICATE STUDENT'S NAME, MATRIC NO. AND FACULTY) hereby declare that the work entitled Profiling of protein involved in Trunking and Non-trunking Sago Palm (TITLE) is my original work. I have not copied from any other student's work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

27 June 2014
Date submitted

Name of the student (Matric No.)

Supervisor's Declaration:
I (SUPERVISOR'S NAME) hereby certifies that the work entitled Profiling of protein involved in Trunking and Non-trunking Sago Palm (TITLE) was prepared by the above named student, and was submitted to the "FACULTY" as a * partial/full fulfilment for the conferment of Bachelor of Science with Honours in Resource Biotechnology (PLEASE INDICATE THE DEGREE), and the aforementioned work, to the best of my knowledge, is the said student's work.

Received for examination by: 27/6/2014

(Name of Mend Hippan and Hussain Dekan
Fakulti Sains dan Teknologi Sumber
UNIVERSITI MALAYSIA SARAWAK
94300 Kota Samarahan)
I declare that Project/Thesis is classified as (Please tick (\))

☐ CONFIDENTIAL (Contains confidential information under the Official Secret Act 1972)*

☐ RESTRICTED (Contains restricted information as specified by the organisation where research was done)*

☑ OPEN ACCESS

Validation of Project/Thesis

I therefore duly affirm with free consent and willingly declare that this said Project/Thesis shall be placed officially in the Centre for Academic Information Services with the abiding interest and rights as follows:

• This Project/Thesis is the sole legal property of Universiti Malaysia Sarawak (UNIMAS).
• The Centre for Academic Information Services has the lawful right to make copies for the purpose of academic and research only and not for other purpose.
• The Centre for Academic Information Services has the lawful right to digitalise the content for the Local Content Database.
• The Centre for Academic Information Services has the lawful right to make copies of the Project/Thesis for academic exchange between Higher Learning Institute.
• No dispute or any claim shall arise from the student itself neither third party on this Project/Thesis once it becomes the sole property of UNIMAS.
• This Project/Thesis or any material, data and information related to it shall not be distributed, published or disclosed to any party by the student except with UNIMAS permission.

Student signature: ____________________________  
(Date) 9/7/2014

Supervisor signature: ____________________________

Current Address: ____________________________________________

Notes: * If the Project/Thesis is CONFIDENTIAL or RESTRICTED, please attach together as annexeure a letter from the organisation with the period and reasons of confidentiality and restriction.

[The instrument is duly prepared by The Centre for Academic Information Services]
Table of Content

Acknowledgements ................................................................................................. i
Declaration .............................................................................................................. ii
Table of Content ..................................................................................................... iv
List of Abbreviations ............................................................................................... v
List of Tables and Figure ......................................................................................... vi
Abstract .................................................................................................................. 1
Introduction ............................................................................................................. 2
Literature Review ...................................................................................................... 5
   Metroxylon sagu .................................................................................................... 5
   Trunking and Non-Trunking Metroxylon sagu ....................................................... 6
   Protein from the leaves ......................................................................................... 6
   Protein extraction and precipitation .................................................................... 7
   Quantification method ....................................................................................... 7
   Gel staining ....................................................................................................... 8
Materials and Methods ........................................................................................... 9
   Sample preparation ............................................................................................. 9
   Trichloroacetic acid (TCA) in acetone precipitation ........................................ 9
   Ethanol precipitation ......................................................................................... 10
   Protein quantification ....................................................................................... 10
   Protein separation and staining .................................................................... 11
Results ..................................................................................................................... 12
Discussion ............................................................................................................... 19
Conclusion ............................................................................................................... 23
References ............................................................................................................. 24
List of Abbreviations

%  percent
°C  degree celsius
2-D  2-Dimensional
DNA  Deoxyribonucleic acid
F (DFn,DFd)  F (degrees of freedom numerator, degrees of freedom denominator)
g  gram
IEF  Isoelectric focusing
M. sagu  Metrosylon sugu
min  minute
ml  millilitre
mRNA  messenger Ribonucleic acid
NaOH  Sodium hydroxide
nm  nanometre
RNA  Ribonucleic acid
rpm  revolutions per minute
SDS  sodium dodecyl sulfate
SDS-PAGE  sodium dodecyl sulfate polyacrylamide gel electrophoresis
TCA  Trichloroacetic acid
Tris-HCl  Tris-Hydrochloride
UV  ultraviolet
μg  microgram
μg ml  microgram per millilitre
List of Tables and Figure

**Table 1:** Comparison of two precipitation method with Coomassie Brilliant Blue R-250 staining referring to Figure 1A. Protein yield using Bradford protein assay.

**Table 2:** Comparison of two precipitation method with Silver staining referring to Figure 1B. Protein yield using Bradford protein assay.

**Table 3A:** Average protein quantification using Bradford protein assay.

**Table 3B:** Average protein quantification using BCA protein assay.

**Table 4A:** Anova results between TCA in acetone and ethanol precipitation method using Bradford protein assay.

**Table 4B:** Anova results between TCA in acetone and ethanol precipitation method using BCA protein assay.

**Table 4C:** Anova results between quantification method (Bradford protein assay and BCA protein assay) for TCA in acetone precipitation method.

**Table 4D:** Anova results between quantification method (Bradford protein assay and BCA protein assay) for Ethanol precipitation method.

**Figure 1A:** SDS-PAGE run on 12 % gels of protein samples extracted using different methods. Equal amount of protein (10 µg) was loaded in each lane. Proteins were visualized with Coomassie Brilliant Blue R-250.

**Figure 1B:** SDS-PAGE run on 12 % gels of protein samples extracted using different methods. Equal amount of protein (10 µg) was loaded in each lane. Proteins were visualized with Silver staining.

**Figure 2A:** SDS-PAGE run on 12 % gels of protein samples extracted using different methods. Equal amount of protein (10 µg) was loaded in each lane. Proteins were visualized with Coomassie Brilliant Blue R-250.

**Figure 2B:** SDS-PAGE run on 12 % gels of protein samples extracted using different methods. Equal amount of protein (10 µg) was loaded in each lane. Proteins were visualized with Coomassie Brilliant Blue R-250.

**Figure 3A:** Standard curve for Bradford protein assay.

**Figure 3B:** Standard curve for Bicinchoninic Acid (BCA) protein assay.

**Figure 4:** SDS-PAGE run on 12 % gels of protein samples extracted using different methods. Equal amount of protein (10 µg) was loaded in each lane. Proteins were visualized with Coomassie Brilliant Blue R-250 staining. Lane 1, 3, 5 and 7 (counted after the protein ladder) was protein extracted using Phenol method. Lane 2, 4, 6 and 8 was protein extracted using acetone method.
Abstract

*Metroxylon sagu* is recognised as an important crop in this 21st century. Abnormalities of this crop growth contribute to the existence of a non-trunking *Metroxylon sagu*. This existence gives curiosity rise in the differences of genetic makeup in both trunking and non-trunking *Metroxylon sagu*. Thus, the objective of this study is to identify protein involved in both trunking and non-trunking sago palm by using different precipitation method screening on SDS-PAGE gel electrophoresis. Modified Trichloroacetic acid (TCA) in acetone precipitation method and Ethanol precipitation method were used for the precipitation followed by the SDS-PAGE electrophoresis analysis for protein separation comparison. The identified protein gained from SDS-PAGE was compared for protein separation differences. As the result, Ethanol precipitation shows better protein yield quantificationally but TCA in acetone precipitation give better image range in the protein analysis by SDS-PAGE gel electrophoresis. This shows that TCA in acetone as a better precipitation method for *Metroxylon sagu* extraction.

**Keywords:** *Metroxylon sagu*, trunking, non-trunking, protein extraction, method comparison

Abstrak


*Kata kunci:* *Metroxylon sagu*, berbatang, tiada-batang, pemendakan protein, perbandingan kaedah
Introduction

*Metroxylon sagu* or commonly known as sago palm is cultivated for its rich starch trunk (Avé, 1977). This sago starch can be produced into variety of production. Thus, the trunk of the sago palm is the most precious part. Unfortunately, the stunted growth sago palm affected the starch cultivation as the stunted growth parts are generally occurred in the trunk of the palm. This atypical growth of sago becomes the main focus for many researchers that are interested in the differences of physical and biological growth of the sago. Roughly, these differences may be varied because of the presences or absences of some proteins or may be in the genetic material. Thus, the identification of protein involved in both trunk and non-trunking may verify this statement.

For the records, most research involving *Metroxylon sagu* are all about the starch capacity. Most of the research are focused on starch debranching, sugar developing from starch, sago hampas and even the transplant of sago before the trunking periods. This shows less information about the genetic material of *Metroxylon sagu*. Fortunately, there is a study focused on the salt mechanism that resulted on the effect of salt stress on the sago growth ability (Ebara et al., 2013). This research progressively helps in the study of the stunted growth sago palm. Impressively, the very utmost research of the sago palm is the bioethanol production from sago hampas (Awg-Adem et al., 2012). This discovery helps much in green campaign as the discovery are one of the new renewable energy.

In this study, one of the focuses is in extracting the protein from the leaves. Proteins are more preferable in this identification study because protein establish particular biological activity. From this protein extraction and purification, valuable things could be recognized. Apart from that, proteins are the key component that determines gene expression and mRNA translations machinery (Ehle et al., 1989). This helps in the leads of the differences between
trunking and non-trunking sago palm. From this step, the study can be further analysed by identifying the specific protein structure and even metabolism pathway that causes the differences in the growth metabolism. However, before the study can further be continued, the basic step of protein extraction plays the crucial role in the protein preparation. As we know, plants cell wall thickness range from 5 - 80 nm because plants cell needs to protect their cell from dryness and infections (Cosgrove, 2005). This thickness became one of the boundaries in protein extraction. Thus, the right protein extraction method should be used in overcoming this obstacle.

The second focus is in protein precipitate formation. This formation occurs in a chronic process. This process consist of (1) mixing phase for precipitant and protein collision, (2) nucleation phase for protein aggregation generation and lastly (3) aging phase where protein particles stabilize to withstand fluid shearing (Zellner et al., 2005). The first phase involved only the introduction of precipitant to the protein solution for the present of collision between both particles. This collision continues to nucleation phase where protein masses are generated through flocculation. This phase take more time since it has slower rate. While the final phase involve breakup of the particle to reach stable state where the protein particle can resist the high fluid shear force. The protein precipitation formation followed by salting out for protein-protein interaction. This step causes the protein to aggregate, precipitate and normalize the protein.

In order to study protein, the protein must be separated to recognize the broad range of the protein according to their sizes. This help in the initial step of the protein study. Appropriate protein detection method is highly essential because the protein study approach measures the quantitative changes in the expression levels in the biological samples. For the basic instrument, SDS-PAGE electrophoresis is used. This technique is used because the
Literature Review

*Metroxylon sagu*

*Metroxylon sagu* or commonly known as sago palm, originated from Javanese, sago means palm pith that contains starch. *Metroxylon sagu* belongs to Arecales Nakai order in Palmae Jussieu family in Calamoideae Griffith subfamily in Metroxylinae Blume sub-tribe in Metroxylon Rutthuell genus (Heywood, 1993). The generic name for *Metroxylon* is a Greek that means the pith of the tree wood (Orwa et al., 2009). Different to the other *Metroxylon* species, *Metroxylon sagu* has 18 scales rows on the fruits and massive robust size of the inflorescent branches (McClatchey et al., 2006). Briefly, *Metroxylon sagu* is a true sago palm without leaf sheaths that have a trunk in the size of 30-60 cm diameters and a height of 6-16 m. Sago palm is a pinnate-leaved tree and also hapaxanthic (Orwa et al., 2009). There is four growing stage for *Metroxylon sagu*: rosette stage, bole formation stage, inflorescence stage, and fruit ripening stage (Flach, 1997). After 4-6 years, the seedling of *Metroxylon sagu* grows into the rosette stage where the leaves and trunks are formed (Jong, 1995). In bole formation stage, the trunk grows to the length of 6-14 m and reach an optimum crown leaves of 24 (Flach, 1997). Formation of inflorescence stage (after 4-14 years of trunk formation) is the stage where the store starch is used (Jong, 1995). When mature (where the starch fills the trunk to the crown), the primary axis divide the secondary and tertiary axes (Karim et al., 2008). According to Flach (1977), the fruiting occur at the tertiary axes. This development last for about 2 years. Compare to the other *Metroxylon*, *Metroxylon sagu* reproduced by vegetative sucker and often by stolon (McClatchey et al., 2006).
Trunking and Non-Trunking *Metroxylon sago*

A normal growth sago palm is a palm with starch full trunk when reach the maturity stage that is also known as trunking *Metroxylon sago* (sago palm). While, an abnormal growth sago palm is a palm that have stunted growth and development that is also known as non-trunking *Metroxylon sago* (sago palm). This trunk formation are influenced by the peat depth (Fong et al., 2005). A shallow peat area is the best condition for trunking sago palm. Whereas, the non-trunking sago palm are usually found in the deep soil area. Trunking sago palm needs extra moist soil to fulfil the nutrient content and to have a better compactness. But, a less humidified soil causes stunted growth of the sago palm that eventually deprived the capability to develop bole part (Fong et al., 2005). This poor nutrient growth medium causes the slow growth in palm with premature branch desiccation, slim crowns, minor trunks and very little starch yield.

Protein from the leaves

Protein located from the leaves of the plants is easier to extracted compare to the trunk and the root part. Marsoni et al. (2005) stated that the leaves part is the easiest part to grind and this part give higher extraction yield compare to the roots. While, compare the leaves and the trunk part, protein extraction from the leaves is easier to extract compare to the trunk because of the high level of starch in the trunk. For *Metroxylon sago* in both trunking and non-trunking sago palm, the expression of protein may be differ. Thus, the proteomic analysis is done to identify the differences. As stated by Hussain et al. (2012), several level of protein has been detected and successfully differentiated from both trunking and non-trunking by using 2 different method and analyse by using 2D-gel electrophoresis. The protein extracted from the leave can also be first detected using SDS-PAGE electrophoresis before proceeding to 2D-gel electrophoresis.
Protein extraction and precipitation

Briefly, there are several techniques for protein extraction. Many different techniques have been developed because every protein is different. Different techniques had been used to achieve the best possible yield and purity from different type of species of an organism, different sample types and even different target molecule. The basic method of extraction is by using mortar and pestle. With the assist from liquid nitrogen, this extraction became easier. Liquid nitrogen acts as freeze medium to ease the cell disruption. The liquid nitrogen also can be replaced with detergent-based lysis buffer. This cell disruption step act as the first step in organelle isolation, cell fractionation, protein isolation and purification (Moré et al., 1994).

Followed by the cell disruption for protein is protein precipitation. This step is to concentrate or to remove interferences before doing electrophoresis or even protein determination. This is the principle step in proteomics (Jiang et al., 2004).

Quantification method

Quantification method or known as protein assay method are divided into copper-based protein assay and dye-based protein assay. Copper-based protein assay involve protein-copper chelation in copper reduction and dye-based protein assay involve the binding of color change bounded dye (Bradford, 1976; Seevaratnam et al., 2009). Example for copper-based protein assay are; Peptides and the Biuret Reaction, Bicinchoninic Acid (BCA) protein assays and Lowry protein assays. While, dye-based protein assay are Coomassie Dye (Bradford) protein assays and Pierce 660 nm protein assay. Each assay has its own benefits and limitation. Thus, selecting appropriate method is crucial to a specific sample and for precise estimation results.
Proteins extracted are separated using electrophoresis. This protein bands can be visualized by using different staining methods for detection. This staining technique improved in terms of sensitivity over decades. Staining method is listed as Coomassie Dye staining, Silver staining, Zinc staining, Fluorescent Dye staining and Functional Group-Specific staining. Each of this staining method has its own particular advantages and disadvantages. But, the common of all of this staining method are the incubation steps. All of the staining method must have this four basic steps; (1) water-wash step that roles as the remover of electrophoresis buffers from the gel matrix, (2) an acid- or alcohol-wash step for gel fixing that will limit the diffusion of protein bands, (3) staining step to allow the dye or chemical substance to diffuse into the gel and bind with the proteins and (4) de-staining step to remove excess dye from the background gel matrix (Hayworth, 2009).
Materials and Methods

Sample preparation

The leaves sample of *Metroxylon sagu* was readily prepared. Both the trunking and non-trunking leaves are originated from Dalat, Sarawak. These leaves are stored at -20°C to maintain their freshness. Both of these leaves were cut into smaller pieces with removed midriff. Using cold mortar and pestle with added liquid nitrogen, these leaves were grind into fine tissues powder.

Trichloroacetic acid (TCA) in acetone precipitation

This was a modified publish TCA protocol (Caruso et al., 2009). Tissue powder (0.5 g) was dissolved in 1 ml of ice-cold extraction buffer (0.175 M Tris-HCl; pH 8.8, 0.173 M SDS, 15% glycerol, 0.07% 2-mercaptoethanol). The cell debris was removed by centrifuging at 13,600 rpm at 4°C for 15 min. The supernatant was transferred into a new tube. Four times volume of ice-cold precipitation buffer (acetone containing 10% Trichloroacetic acid and 0.07% 2-mercaptoethanol) was added. The mixtures were stored at -20°C overnight. The pellets were obtained by centrifugation at 13,600 rpm at 4°C for 20 min. Then washed with 20% acetone with 0.07% 2-mercaptoethanol followed by centrifugation at 13,600 rpm at 4°C for 5 min. The supernatant were discarded and the pellets were air dried.
**Ethanol precipitation**

This was modified Ethanol protocol (Lebendiker, 2002). Tissue powder (0.5 g) was dissolved in 1 ml of ice-cold extraction buffer (0.175 M Tris-HCl; pH 8.8, 0.173 M SDS, 15% glycerol, 0.07% 2-mercaptoethanol). The cell debris was removed by centrifuging at 13,600 rpm at 4 C for 15 min. The supernatant was transferred into a new tube. Four times volume of ice-cold 99.98% ethanol with 0.07% 2-mercaptoethanol was added for protein precipitation. The mixture were incubated overnight at -20°C. The pellets were obtained by centrifugation at 13,600 rpm at 4 C for 20 min. Then the pellets were washed with 99.98% ethanol followed by centrifugation at 13,600 rpm at 4 C for 5 min. The supernatant were discarded and the pellets were air dried.

**Protein quantification**

Protein concentration was quantified by using two techniques; Bradford protein assay and BCA protein assay (Bradford, 1976). Both methods used BSA as a standard with six different dilutions and quantified by using UV spectrophotometer (UVmini-1240).

1 ml of Bradford reagent (Coomassie Brilliant Blue G-250, methanol, phosphoric acid) was added to the sample and incubated for 5 min. The mixture was measured at the absorbance of 595 nm.

1 ml of BCA reagent (Reagent A: Bicinchoninic acid, Sodium Carbonate, Sodium Tartrate, Sodium Bicarbonate in NaOH and Reagent B; Copper (II) Sulphate penta-hydrate) was added to the sample and incubated at 60 C for 15 min. The mixture was measured at the absorbance of 562 nm.
Protein separation and staining

SDS-PAGE electrophoresis was performed according to Laemmli (1970). The separating gel was prepared in 12% of polyacrylamide content and stacking gel with 5% of polyacrylamide content. The protein ladder used was in the range of 7 - 175 kDa. Each gel stack was stained using two different staining method; Coomassie Brilliant Blue R-250 staining and Silver staining (Kawasaki et al., 1990; Wray et al., 1981).

For Coomassie Brilliant Blue R-250 staining, the gel was soaked with staining solution (Coomassie R-250, Methanol, Acetic acid) overnight. The gel was then de-stained with de-staining solution (Methanol, Acetic acid) twice for 30 min and 10 min respectively.

For Silver staining, the gel was fixed with fixing solution (Methanol, Formaldehyde, Sodium Thiosulfate) overnight. The gel was then sensitized with sensitizing solution (Sodium Thiosulfate) to increase the stain sensitivity and contrast followed by staining with 0.1% Silver Nitrate solution. The band was then visualized after soaking in the developing solution (Sodium Carbonate, Sodium Thiosulfate, Formaldehyde) for several of seconds.
Results

In this study, two different protein extraction methods were valued for this protein study of *Metroxylon sagu*. The main highlight was set on each extraction method, the gel background, and protein band visualization in terms of smearing and separation (Table 1, Table 2). For precipitation using TCA in acetone precipitation method, an average protein yield for trunking *M. sagu* of 0.633 µg/ml and non-trunking *M. sagu* of 0.624 µg/ml were obtained from Bradford protein assay. Whereas, precipitation using Ethanol precipitation method, an average protein yield for trunking *M. sagu* of 1.213 µg/ml and non-trunking *M. sagu* of 1.225 µg/ml were obtained also using Bradford protein assay. These protein yields comparisons showed that Ethanol precipitation method gave greatest yield for the trunking and non-trunking *M. sagu* among the two precipitation methods using Bradford protein assay. While in BCA protein assay, precipitation using TCA in acetone precipitation method gave an average protein yield for trunking *M. sagu* of 3.062 µg/ml and non-trunking *M. sagu* of 2.832 µg/ml were obtained. Whereas, precipitation using Ethanol precipitation method, an average protein yield for trunking *M. sagu* of 6.911 µg/ml and non-trunking *M. sagu* of 6.923 µg/ml were obtained. These protein yields comparisons also showed that Ethanol precipitation method gave greatest yield for the trunking and non-trunking *M. sagu* among the two precipitation methods using BCA protein assay.
Table 1: Comparison of two precipitation method with Coomassie Brilliant Blue R-250 staining referring to Figure 1A. Protein yield using Bradford protein assay.

<table>
<thead>
<tr>
<th></th>
<th>TCA Trunking</th>
<th>TCA Non-trunking</th>
<th>Ethanol Trunking</th>
<th>Ethanol Non-trunking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein yield</td>
<td>0.633</td>
<td>0.624</td>
<td>1.213</td>
<td>1.225</td>
</tr>
<tr>
<td>Smearing</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Low smear</td>
</tr>
<tr>
<td>Separation</td>
<td>Good</td>
<td>Good</td>
<td>Poor</td>
<td>Poor</td>
</tr>
</tbody>
</table>

Table 2: Comparison of two precipitation method with Silver staining referring to Figure 1B. Protein yield using Bradford protein assay.

<table>
<thead>
<tr>
<th></th>
<th>TCA Trunking</th>
<th>TCA Non-trunking</th>
<th>Ethanol Trunking</th>
<th>Ethanol Non-trunking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein yield</td>
<td>0.633</td>
<td>0.624</td>
<td>1.213</td>
<td>1.225</td>
</tr>
<tr>
<td>Smearing</td>
<td>Low smear</td>
<td>Low smear</td>
<td>Big smear</td>
<td>Big smear</td>
</tr>
<tr>
<td>Separation</td>
<td>Good</td>
<td>Good</td>
<td>Poor</td>
<td>Poor</td>
</tr>
</tbody>
</table>

SDS-PAGE protein profiles with two different staining methods were used to compare both of the precipitation method. Using standard Coomassie Brilliant Blue R-250 staining method, precipitation using TCA in acetone precipitation method produced better resolution of protein separation, lower smearing and clearer background (Figure 1A, 2A, 2B, 2C). While precipitation using Ethanol precipitation method also gives protein separation but poorer in resolution and it also gives average to no smearing (Figure 1A2A, 2B, 2C). Meanwhile staining using Silver staining method showed that both methods produced average protein separation. The precipitation using TCA in acetone precipitation method produced uniform protein separation compare to the Ethanol precipitation method (Figure 1B). In term of smearing, TCA in acetone precipitation method gives lesser smear compare to Ethanol precipitation method. From these results, precipitation with TCA in acetone precipitation method shows the most efficient protein precipitation method compare to Ethanol precipitation for M. sagu protein extraction in the application of proteomic studies.
Figure 1A: SDS-PAGE run on 12 % gels of protein samples extracted using different methods. Equal amount of protein (10 μg) was loaded in each lane. Proteins were visualized with Coomassie Brilliant Blue R-250 staining. (T: Trunking; T²: Trunking with two time dilution; NT: Non-Trunking; NT²: Non-Trunking with two time dilution.)

Figure 1B: SDS-PAGE run on 12 % gels of protein samples extracted using different methods. Equal amount of protein (10 μg) was loaded in each lane. Proteins were visualized with Silver staining. (T: Trunking; T²: Trunking with two time dilution; NT: Non-Trunking; NT²: Non-Trunking with two time dilution.)
Figure 2A: SDS-PAGE run on 12% gels of protein samples extracted using different methods. Equal amount of protein (10 µg) was loaded in each lane. Proteins were visualized with Coomassie Brilliant Blue R-250 staining. (T: Trunking; T*: Trunking with two time dilution; NT: Non-Trunking; NT*: Non-Trunking with two time dilution.)

Figure 2B: SDS-PAGE run on 12% gels of protein samples extracted using different methods. Equal amount of protein (10 µg) was loaded in each lane. Proteins were visualized with Coomassie Brilliant Blue R-250 staining. (T: Trunking; T*: Trunking with two time dilution; NT: Non-Trunking; NT*: Non-Trunking with two time dilution.)
Figure 3A: Standard curve for Bradford protein assay quantification.

Figure 3B: Standard curve for Bicinchoninic Acid (BCA) protein assay.

Table 3A: Average protein quantification using Bradford protein assay.

<table>
<thead>
<tr>
<th></th>
<th>TCA</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trunking</td>
<td>0.633</td>
<td>1.213</td>
</tr>
<tr>
<td>Non-trunking</td>
<td>0.624</td>
<td>1.125</td>
</tr>
</tbody>
</table>

Table 3B: Average protein quantification using BCA protein assay.

<table>
<thead>
<tr>
<th></th>
<th>TCA</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trunking</td>
<td>3.063</td>
<td>6.911</td>
</tr>
<tr>
<td>Non-trunking</td>
<td>2.363</td>
<td>6.923</td>
</tr>
</tbody>
</table>
### Table 4A: Anova results between TCA in acetone and ethanol precipitation method using Bradford protein assay.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of Sago palm (a)</td>
<td>7.04E-06</td>
<td>1</td>
<td>7.04E-06</td>
<td>0.0008</td>
<td>0.978</td>
<td>5.318</td>
</tr>
<tr>
<td>Precipitation method (b)</td>
<td>1.047</td>
<td>1</td>
<td>1.047</td>
<td>118.370</td>
<td>4.51E-06</td>
<td>5.318</td>
</tr>
<tr>
<td>Interaction (a x b)</td>
<td>0.00034</td>
<td>1</td>
<td>0.00034</td>
<td>0.039</td>
<td>0.848</td>
<td>5.318</td>
</tr>
<tr>
<td>Within</td>
<td>0.071</td>
<td>8</td>
<td>0.00885</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4B: Anova results between TCA in acetone and ethanol precipitation method using BCA protein assay.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of Sago palm (a)</td>
<td>0.355</td>
<td>1</td>
<td>0.355</td>
<td>0.727</td>
<td>0.419</td>
<td>5.318</td>
</tr>
<tr>
<td>Precipitation method (b)</td>
<td>53.029</td>
<td>1</td>
<td>53.029</td>
<td>108.654</td>
<td>6.22E-06</td>
<td>5.318</td>
</tr>
<tr>
<td>Interaction (a x b)</td>
<td>0.380</td>
<td>1</td>
<td>0.380</td>
<td>0.780</td>
<td>0.403</td>
<td>5.318</td>
</tr>
<tr>
<td>Within</td>
<td>3.904</td>
<td>8</td>
<td>0.488</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4C: Anova results between quantification method (Bradford protein assay and BCA protein assay) for TCA in acetone precipitation method.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantification method (a)</td>
<td>13.030</td>
<td>1</td>
<td>13.030</td>
<td>254.421</td>
<td>2.39E-07</td>
<td>5.318</td>
</tr>
<tr>
<td>Type of Sago palm (b)</td>
<td>0.377</td>
<td>1</td>
<td>0.377</td>
<td>7.366</td>
<td>0.026</td>
<td>5.318</td>
</tr>
<tr>
<td>Interaction (a x b)</td>
<td>0.358</td>
<td>1</td>
<td>0.358</td>
<td>6.989</td>
<td>0.030</td>
<td>5.318</td>
</tr>
<tr>
<td>Within</td>
<td>0.410</td>
<td>8</td>
<td>0.051</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4D: Anova results between quantification method (Bradford protein assay and BCA protein assay) for Ethanol precipitation method.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantification method (a)</td>
<td>97.388</td>
<td>1</td>
<td>97.388</td>
<td>218.516</td>
<td>4.32E-07</td>
<td>5.318</td>
</tr>
<tr>
<td>Type of Sago palm (b)</td>
<td>0.000</td>
<td>1</td>
<td>0.0004</td>
<td>0.001</td>
<td>0.975</td>
<td>5.318</td>
</tr>
<tr>
<td>Interaction (a x b)</td>
<td>1.51E-09</td>
<td>1</td>
<td>1.51E-09</td>
<td>3.39E-09</td>
<td>1.000</td>
<td>5.318</td>
</tr>
<tr>
<td>Within</td>
<td>3.565</td>
<td>8</td>
<td>0.446</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 4: SDS-PAGE run on 12% gels of protein samples extracted using different methods. Equal amount of protein (10 μg) was loaded in each lane. Proteins were visualized with Coomassie Brilliant Blue R-250 staining. Lane 1, 3, 5 and 7 (counted after the protein ladder) was protein extracted using Phenol method. Lane 2, 4, 6 and 8 was protein extracted using acetone method.

(Contributed by: Zulaikha Binti Pol Ong)