

EFFECT OF α -AMYLASE PRETREATMENT ON PROTEIN EXTRACTION FROM DEFATTED ROSELLE SEED

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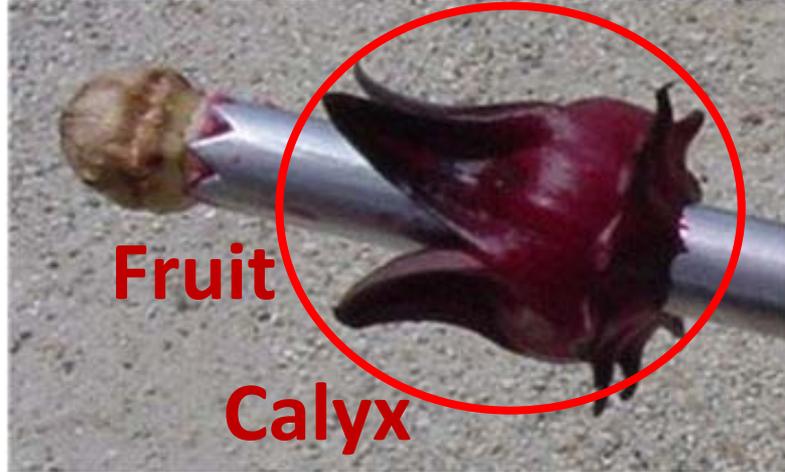
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Outline

- ❖ Introduction
- ❖ Objectives
- ❖ Materials and methods
- ❖ Results and discussion
- ❖ Conclusion

INTRODUCTION

Roselle seed (*Hibiscus sabdariffa* Linn)



Roselle seed is a **byproduct** in the production of roselle calyx and is normally discarded as **waste** or used as cattle-feed.

Roselle seed (*Hibiscus sabdariffa* Linn)



Roselle seed

- Source of protein (19.1 – 28.1%), lipid (18.8 – 35.4%) and carbohydrate (26.6 – 36.4%).
- Excellent feed for chicken and livestock.
- Ingredient for human meal (seed powder, fermented product)
- Roselle seed protein can be used as supplement food mixture for human and animal, due to its essential amino acids profile.
- Potential of lowering total cholesterol and low density lipoprotein cholesterol levels in rat.
- Found an advantageous to isolate protein from defatted roselle seed by using water and saline solution at pH 9.
- α - Amylase attack bonding of oligosaccharide and protein, which enhance extractable protein.

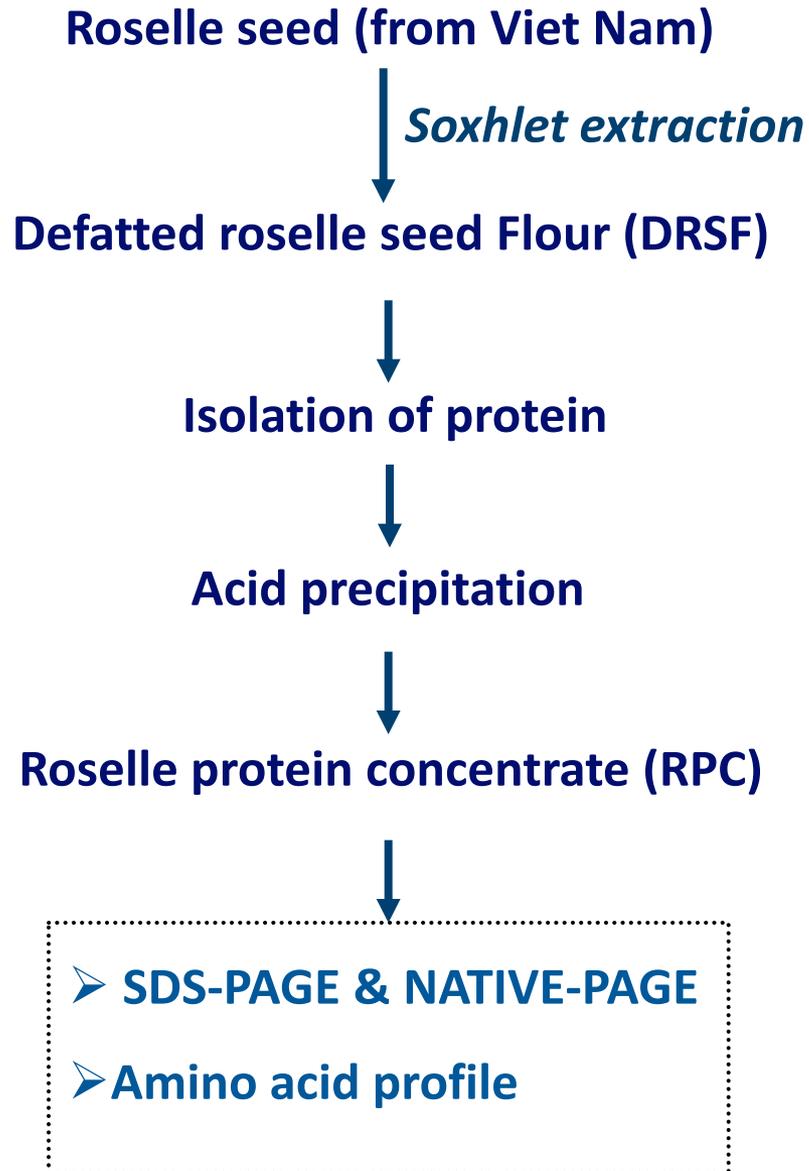
Objectives

Protein extraction with α -amylase pretreatment

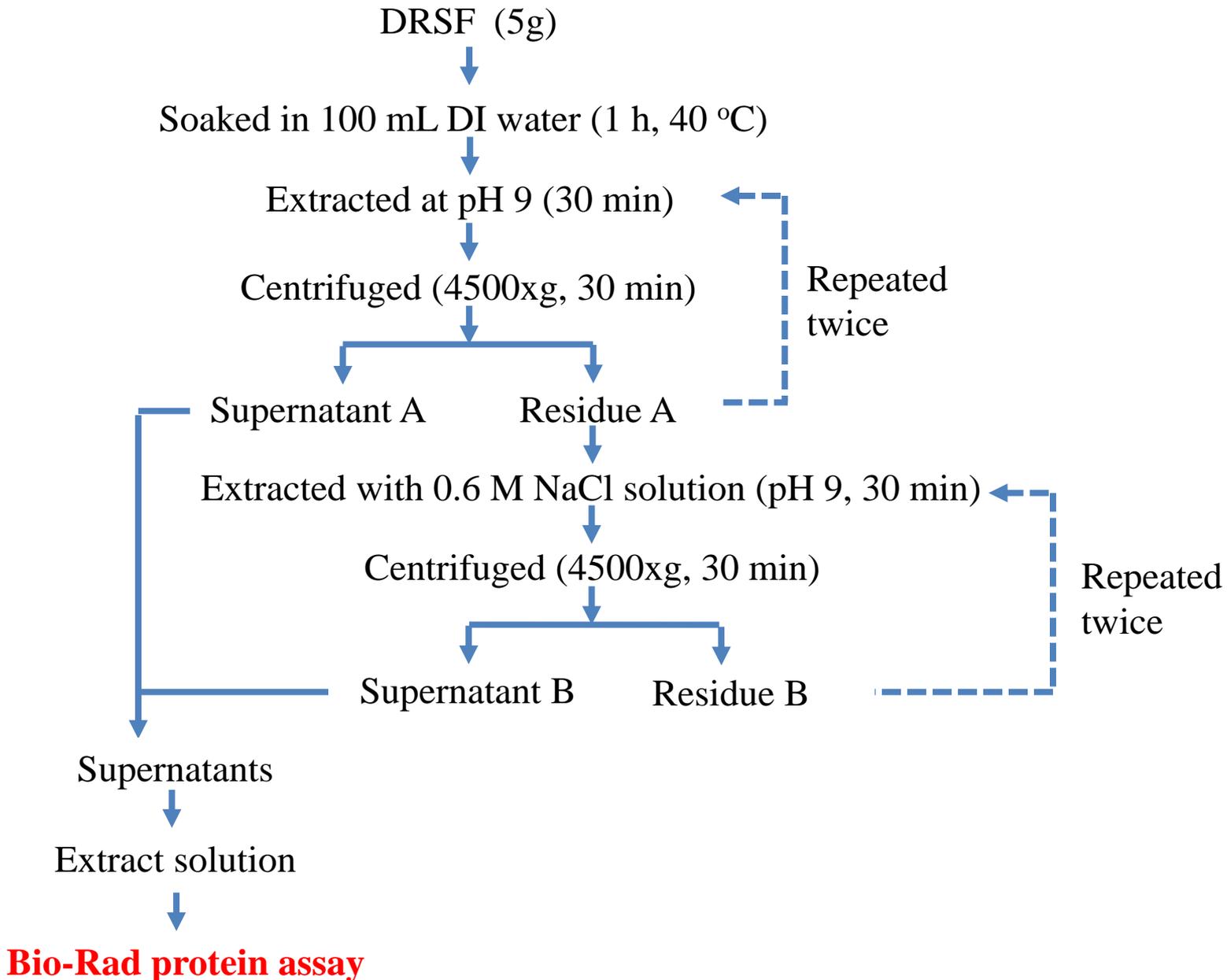
- ⊕ Effect of α -amylase on protein extraction yield
- ⊕ Molecular weight of roselle protein (SDS PAGE)

MATERIALS & METHODS

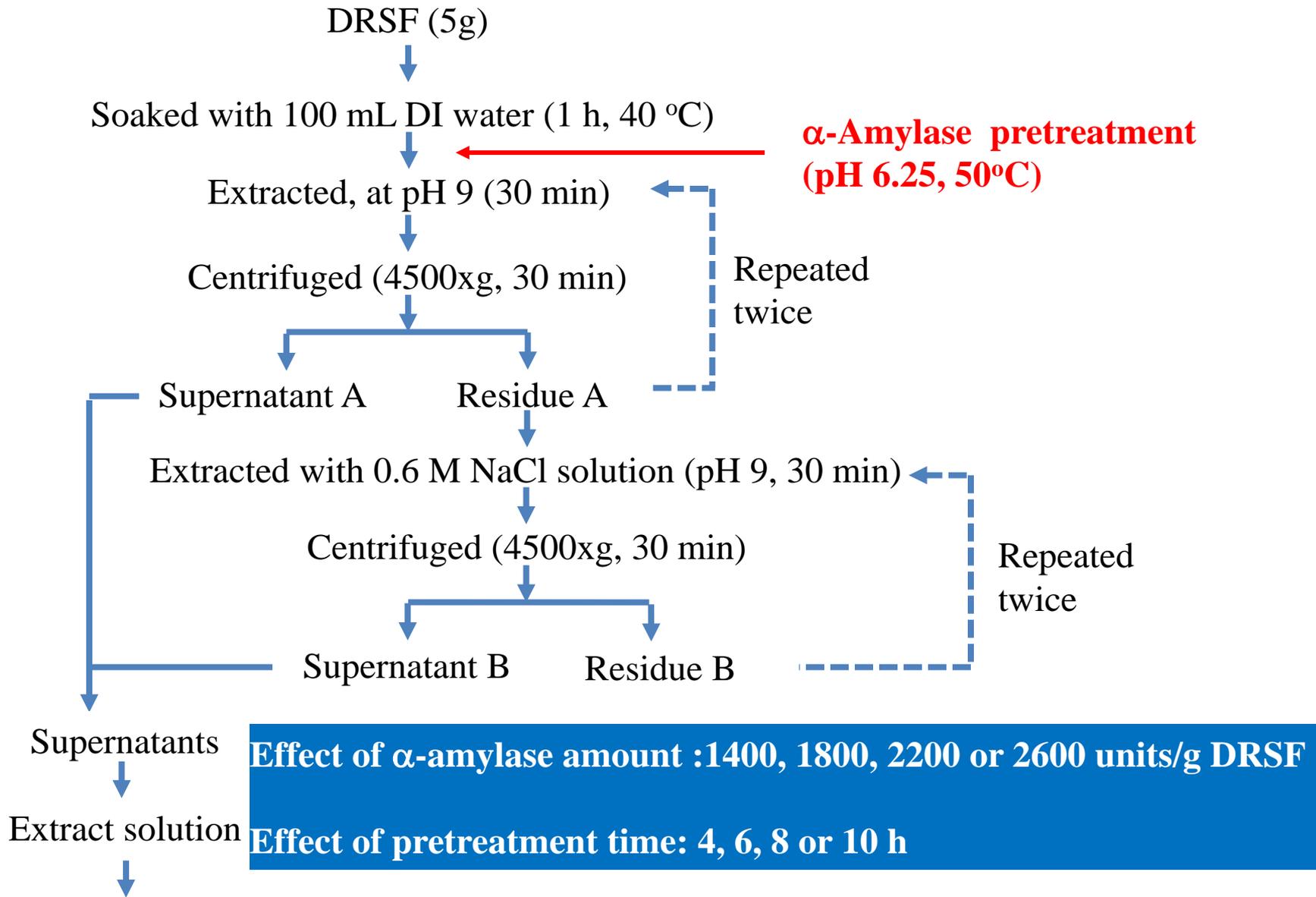
Conceptual structure of study



Protein extraction without α -amylase pretreatment



Protein extraction with α -amylase pretreatment



Bio-Rad protein assay

RESULTS AND DISCUSSION

Yield of protein extraction with and without pH adjustment

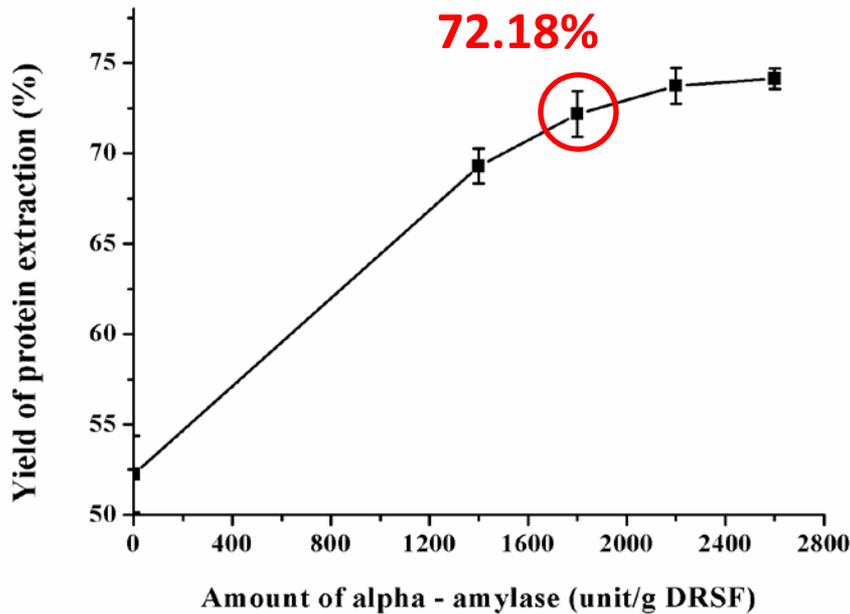
| Solvent | Yield of protein extraction (%) | |
|--------------|---------------------------------|--------------|
| | Without pH adjustment | pH 9 |
| DI water | 20.25±1.47 | 25.32±2.01 |
| NaCl | 26.47±1.32 | 26.92±1.66 |
| Total | 46.72 | 52.24 |

Compositions of DRSF and RPC (%)

| | DRSF | RPC^a |
|----------------|-------------------|------------------------|
| Protein | 38.18±0.20 | 86.99±0.06 |
| Starch | 22.25±0.18 | 3.61±0.11 |
| Fiber | 27.92±0.11 | 4.22±0.10 |
| Ash | 8.50±0.04 | 4.90±0.06 |

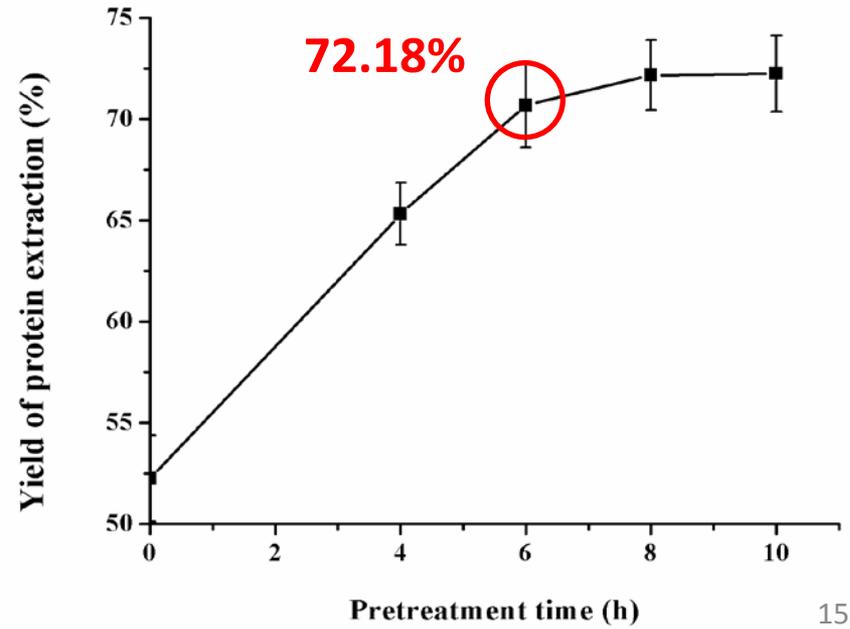
^a The composition was measured from RPC, which was obtained in optimum condition of α -amylase pretreatment (1 800 units α -amylase/g DRSF; 6 h hydrolysis time)

Effect of α -amylase amount and pretreatment time on protein yield

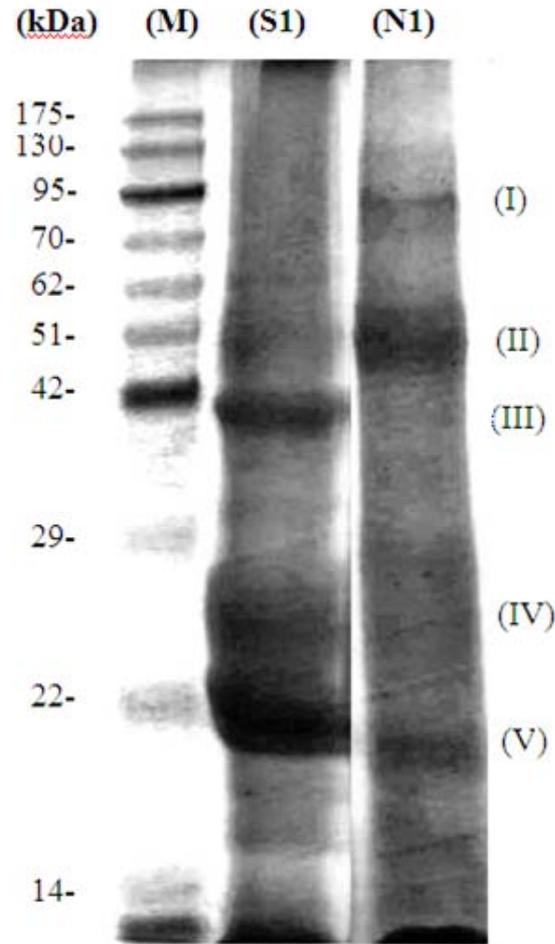


**Amount of α -amylase
= 1800 units/g DRSF**

Pretreatment time = 6 h



Protein molecular weight



Coomassie-stained SDS-acrylamide gel (12% acrylamide) containing RPC samples. M-Marker; S1 - SDS PAGE of RPC; N1 – native PAGE of RPC.

Amino acids compositions (%) of RPC, protein isolate obtained from previous study^a (Al-Numair & Ahmed , 2008)

| Amino acids composition | RPC | Roselle seed protein isolate ^a |
|-------------------------|------------|---|
| Essential | | |
| Lysine | 3.84±0.13 | 5.10±0.41 |
| Threonine | 4.24±0.29 | 2.91±0.28 |
| Valine | 4.37±0.39 | 4.55±0.02 |
| Methionine+Cystine | NA | 3.89 |
| Methionine | 0.99±0.31 | 1.48±0.01 |
| Cystine | NA | 2.41±0.09 |
| Isoleucine | 3.25±0.23 | 3.01±0.17 |
| Leucine | 6.36±0.27 | 5.92±0.50 |
| Phenylalanine+Tyrosine | NA | 8.71 |
| Phenylalanine | 4.16±0.28 | 5.99±0.29 |
| Tyrosine | NA | 2.72±0.26 |
| Histidine | 2.35±0.19 | 1.80±0.18 |
| Tryptophan | NA | 0.76±0.18 |
| Non-essential | | |
| Arginine | 10.21±0.34 | 9.58±0.26 |
| Aspartic acid | 10.11±0.56 | 10.28±0.29 |
| Glutamic acid | 28.78±0.50 | 24.00±0.59 |
| Proline | 4.29±0.17 | 4.30±0.20 |
| Glycine | 4.96±0.06 | 5.09±0.21 |
| Alanine | 4.05±0.36 | 5.56±0.06 |
| Serine | 5.51±0.25 | 4.70±0.21 |

Conclusion

- ✿ α -Amylase pretreatment was effective in enhancing protein extraction yield from DRSF (from 52.24% to 72.18%)
- ✿ Pretreatment time has a slight effect on protein yield, whereas protein yield strongly depends on the amount of α -amylase used
- ✿ The major roselle proteins is high molecular weight protein
- ✿ Methionine is the limiting amino acid of protein obtained from roselle seeds cultivated in Vietnam

ACKNOWLEDGEMENT

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Thank you for your attention!