AJChE 2019, Vol. 19, No. 2, 147 – 154

Hydrolytic Process of Proteins in *Moringa oleifera* Seeds in Varied Concentrations of Sodium Hydroxide and Hydrochloric Acid

Ika Kurniaty*
Ratri Ariatmi Nugrahani
Fatma Sari
Wenny Diah Rusanti
Haryadi Wibowo
Department of Chemical Engineering, Engineering Faculty, Universitas Muhammadiyah Jakarta, Jln Cempaka Putih Tengah 27 Jakarta 10510
*Email : ika.kurniaty@ftumj.ac.id

Indonesia is endowed with an immense biodiversity that can be used as protein sources. One of these is *Moringa oleifera* tree that is locally known as Kelor. The seeds of this plant can be used as a protein source, an effective coagulant for water purification, a natural absorbent, and an antimicrobial treatment. Kelor seeds are known to contain fibers, proteins, carbohydrates, and vitamins. The objectives of this study were to identify the optimal solution concentration, determine the yield percentage, and determine the optimal protein content from hydrolytic processes of Moringa seed extraction using Sodium Hydroxide (NaOH) and Hydrochloric Acid (HCl). The hydrolysis took place for 30 minutes at 60^{o}C. The proteins extracted from Moringa seeds were identified with biuret and Braford tests. The NaOH extractions resulted in the highest yield of 12.1% and protein content of 0.43% with 2% NaOH. Whereas those of HCl produced the highest yield of 11.1% and protein content of 9.63% with 1% HCl.

Keywords : Hydrochloric acid, hydrolysis, protein, sodium hydroxide

INTRODUCTION

Indonesia is blessed with a vast biodiversity that can be utilized as protein sources, and one of these is the *Moringa oleifera* plant. *Moringa* is known to be highly nutritious, and the *World Health Organization* (WHO) has introduced it as an alternative foodstuff to combat malnutrition. This plant has been known to be a source of proteins (Aminah, et al., 2015).

Moringa is the most commonly cultivated species among the monogeneric families. This plant is vast growing and known with many names such as lobak/horseradish tree, drumstick tree, benzolive, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna, minyak Ben tree, etc. Moringa has been widely grown in the tropics and considered a miracle tree because of its various uses from many of its parts,

DOI: 10.22146/ajche.50383

especially the seeds (Bramantyo, et al., 2014; Idris, et al., 2016).

The seeds are round with the initial color of green, which turns into dark brown as the pods mature and dry up (Aminah, et al., 2015). The coat of mature Moringa seeds is semi-permeable and has three soft white wings. Each tree can produce in the average of 15,000 to 25,000 seeds, and each seed weighs in the average of 0.3 gram (Krisnadi, 2015). Moringa seeds can be used effectively as a coagulant in water purification treatment, natural absorbent, and anti-microbial drug (Mangale, et al., 2012).

Moringa seeds as shown on Fig. 1, contain several beneficial nutrients such as proteins, fats, carbohydrates, vitamins A, E, C, B1 to B3, calcium etc. (Sahay, et al., 2017). As shown in Table 1, the ingredients with the highest amount in Moringa seeds are proteins (35.97±0.19 gr) and fats (38.67±0.03 gr), and therefore Moringa seeds are a good candidate to become a secondary protein food source.

One of the methods to obtain the protein content of Moringa seeds is through a hydrolytic process. Through hydrolytic methods, proteins from Moringa seeds can be converted into L-amino acids, nucleotides, and other various peptides. Hydrolytic processes can be performed chemically (by adding acidic or basic solutions) or enzymatically. A chemical hydrolysis process by adding an acid may shorten the time of, reduce the price of, and ease the procedures (Witono, et al., 2007).

The objectives of this study included 1) determining the optimal concentrations of NaOH and HCl, 2) determining the highest yields, and 3) determining the protein contents from hydrolytic extractions of Moringa seeds.

Fig 1. (A) Seedling, (B) Seeds (Leone, et al., 2016)

Table 1. Nutrient Contents per 100 grams of Moringa Seeds (Gopalakrishnan, et al., 2016)

No	Component	Amount
1	Calories (kal)	-
2	Protein (gr)	35.97±0.19
3	Fat (gr)	38.67±0.03
4	Carbohydrate (gr)	8.67±0.12
5	Fiber (gr)	2.87±0.03
6	Vitamin B1(mg)	0.05
7	Vitamin B2(mg)	0.06
8	Vitamin B3(mg)	0.2
9	Vitamin C (mg)	4.5±0.17
10	Vitamin E (mg)	751.67±4.41
11	Calcium (mg)	45
12	Magnesium (mg)	635±8.66
13	Phosphorus (mg)	75
14	Potassium (mg)	-
15	Copper (mg)	5.20±0.15
16	Iron (mg)	-
17	Sulfur (mg)	0.05

RESEARCH METHOD

The research methodology was divided into these stages: material preparation, hydrolytic processes, and analytical procedures.

Material Preparation

Moringa seeds were washed and dried in an oven at 100^{o}C to reduce the water content. The dried seeds were ground into powder.

Hydrolytic Processes

The Moringa seed powder was placed into a three-neck flask that had been equipped with liebig cooler, thermometer, stirrer, and heater. The solutions used in this study were Sodium Hydroxide (NaOH) and Hydrochloric Acid (HCl). The concentrations tested in this study were (2, 4, 6, 8, and 10) % (w/v) of NaOH and (0.2, 0.4, 0.6, 0.8, and 1.0) % (v/v) of HCl. The dispersed Moringa seed powder was stirred and heated at low temperature (60^{o}C) for 30 minutes to minimize protein denaturation.

The hydrolysis results were settled and filtered to separate the residue from the filtrate. The solid residue was dried up and analyzed for protein contents. The pH of the liquid filtrate neutralized. Once the trial filtrates reached pH 4.7 (pH of acidic conditions) (Naga, Adiguna, Retnoningtyas & Ayucitra, 2010; Triyono, 2010) and pH 7-9 (pH of base conditions) in order to the protein content in the filtrate coagulated, they centrifuged to separate the pellets from the supernatants for additional protein extraction yield. The pellets were dried and weighed.

Analytical Procedures

The analytical procedures consisted of qualitative analyses using Biuret tests and quantitative analyses using Bradford tests, as well as yield concentration determination.

Qualitative Analyses with Biuret tests

The Biuret reagent was prepared with 0.15 gram of Copper (II) sulphate and 0.6 gram of NaK Tartrate dissolved in 50 mL of distilled water. After the salts completely dissolved, 30 mL of 10% NaOH was added and the volume was brought up to 100 mL with the addition of distilled water (Salim & Rahayu, 2017). The reagent was added to the hydrolyzed samples to detect the presence of protein. A positive result would yield samples with red to purple colors. (Wijaya, Dewi, & Sastrodihardjo, 2013).

Quantitative Analyses with Bradford tests

The Bradford reagent was prepared by dissolving 10 mg of coomassie brilliant blue G-250 in 5 ml of 95% ethanol and adding it with 10ml of 85% phosphoric acid. The solution was brought up to 100 ml with distilled water. 2.5 mL of Bradford reagent was added to 50 mL of hydrolysis samples. After vigorous mixing, the mixture was incubated for 10 minutes. Optical density was read at 595nm (Purwanto and Marianti, 2014).

Determining the Yield Percentage

The equation 1 was used to determine the yield percentage of protein extraction from Moringa seeds using hydrolyses.

$$\frac{Moringa\ seed\ weight\ A\ \ (Protein)(gr)}{Mass\ of\ Moringa\ Seed\ (B)\ (gr)}\ x\ 100\%...(1)$$

where :

A : Protein extracted into NaOH/HCl Solution

B : Mass *Moringa* seed before extracted

RESULTS AND DISCUSSION

The yield percentage was determined by comparing the weight of protein yield with the initial weight of Moringa seed preparation. Moringa seed hydrolysis using different concentrations of Sodium Hydroxide (NaOH, an alkali) produced different yields. The highest yield percentage was 12.1% as a result from 2% NaOH hydrolysis (Table 2). There is a negative correlation between NaOH concentrations and yield percentage, in which higher NaOH concentrations produced lower yield percentages. This correlation can be seen from Fig. 2, in which y-axis represents % yield and x-axis represents NaOH concentration.

Table 2. Yields of Moringa Seed Hydrolysis at Various NaOH Concentrations

NO	NaOH Concentration (%)	Yield (%)
1	2	12.1
2	4	7
3	6	6.1
4	8	4.7
5	10	3.15

The result of Bradford tests to determine protein contents is presented in Table 3 and Mass Balance of *Moringa* seed protein extracted into NaOH is presented in Table 4. The highest protein content in the filtrate was found in the hydrolysis using 2% NaOH at 0.43%, and the lowest was from using 10% NaOH at 0.03% in which higher base concentration produces more protein molecules dissolved in the solution. The higher extraction yield produced higher protein content because there was more protein coagulation (Nurhayati, et al., 2018)

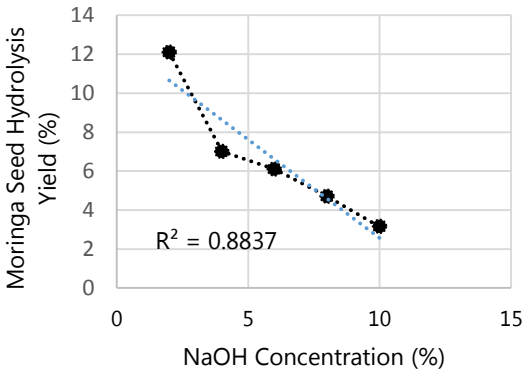

Fig 2. The Effects of NaOH Concentrations on the Yield of Moringa Seed Hydrolysis

Table 3. The Protein Contents in The Filtrate from Moringa Seeds Hydrolyzed with Varied Concentrations of NaOH Solution

No	NaOH Concentration (%)	Protein Content in The Filtrate (%)
1	2	0.43
2	4	0.05
3	6	0.04
4	8	0.03
5	10	0.05

Table 4. Mass Balance of Moringa Seed Protein Extracted into NaOH

No	Total of Moringa Seed Protein (%)	Protein Extracted into NaOH Solution (%)	Protein Remains in The Seed (%)
1	1.77	0.43	1.34
2		0.05	1.72
3		0.04	1.73
4		0.03	1.74
5		0.05	1.72

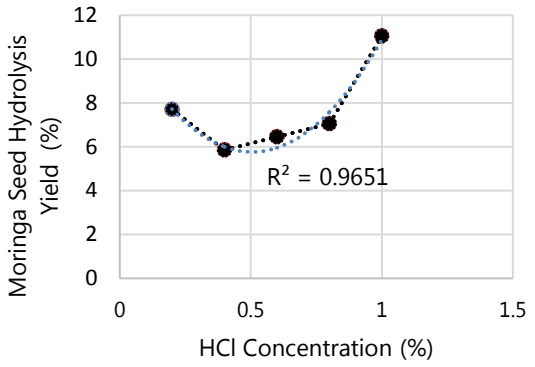

Fig 3.The Effects of HCl Concentrationson the Yield of Moringa Seed Hydrolysis

Table 5. Yields of Moringa Seed Hydrolysis at Various HCl Concentrations

No	HCl Concentration (%)	Yield (%)
1	0.2	7.7
2	0.4	5.85
3	0.6	6.45
4	0.8	7.05
5	1	11.05

Table 6. The Protein Contents in The Filrate from Moringa Seeds Hydrolyzed with Varied Concentrations of HCl

No	HCl Concentration (%)	Protein Content in The Filtrate (%)
1	0.2	1.51
2	0.4	2.96
3	0.6	3.61
4	0.8	2.40
5	1.0	9.63

The other solvent tested was a strong acid, Hydrochloric acid (HCl). These tests produced the highest extraction yield of 11.05% at 1% HCl and the lowest of 5.85% at 0.4% HCl (Table 5). This can be seen from Fig. 3, in which y-axis represents % yield and x-axis represents HCl concentration.

Table 7. Mass Balance of Moringa Seed Protein Extracted into HCl

No	Total of Moringa Seed Protein (%)	Protein Extracted into HCl Solution (%)	Mass Balance of Moringa Seed Protein (%)
1	1.77	1.51	0.26
2		2.96	1.19
3		3.61	1.84
4		2.40	0.63
5		9.63	7.86

Alkaline extraction produced relatively lower protein fraction than acidic one. Furthermore, different temperatures and solvent concentrations affect secondary and tertiary structures of proteins (W. Selling et al., 2013).

Table 6 and Table 7 show that the acidic extraction of Moringa seed produced the highest protein content of 9.63% using 1% HCl. Increasing concentrations of HCl will be increased the rate of hydrolysis because the hydrolysis reaction rate constant is proportional to the concentration of H^+ in acidic conditions (Nofi & Putra, 2013).

Biuret test were applied to analyze the protein contents in the Moringa seed hydrolytic extraction samples. The presence of proteins is marked by the formation of purple or violet color in the samples. This phenomenon can be explained by the reactions that take place between peptides in the samples and Cu^{2+} ions from $CuSO_4$ in the biuret reagent. Purple color formation from biuret tests in Moringa seed hydrolytic extractions indicates the presence of proteins in Moringa seeds (Bakhtra, et al., 2016; Jubaidah, et al., 2016).

According to (Sugiyono, 2004) If the protein heated for several hours in 6N HCl, it will ultimately be hydrolyzed into its amino acid components. If the protein boiled in NaOH 5N, it will also experience complete hydrolysis. This article used different NaOH and HCl concentrations as a solvent so that the results much lower than the expected.

Hydrolysis of proteins with acids will produce amino acids. The resulting amount of amino acids is called free α-amino nitrogen content. During hydrolysis, conversions of insoluble proteins into soluble ones take place, and further breakdowns also occur that result in more simple compounds, such as peptides, amino acids, and ammonia(Kurniawan, et al., 2012; Anggraini & Yunianta, 2015)..

Amino acids which have permanent optical active properties (L-form) as found in nature. Hydrolysis using alkali will change the optically active properties of amino acids due to the presence of racemization (a mixture of L and D forms of amino acids) (Nofi & Putra, 2013) and the different treatment of HCl and NaOH addition in the hydrolysis process will determine the type of amino acid produced (Sugiyono, 2004).

Protein solubility can be increased with acidic treatments because of the positive charge from the acids modifies the previously neutral protein molecules into positively charged ones. Prolonged heating at 50 to 80 ^{o}C and acid addition with high concentration can induce denaturation that results in protein precipitation (Triyono, 2010).

Based on Table 1, the protein should be in the range of 35% but, in this study the protein content in the filtrate is much lower than that. Several factors influenced these results with the existing theories. It can be the weight of Moringa seeds to extracted into the oil, percentage of solvent concentration, type of solvent used, the process to obtain the protein content with hydrolysis or another one, time, and temperature of the protein isolation process, then human errors. Also, the Carbohydrate components in Moringa seed oil can interfere because they are formed humin compounds in the form of particles that can absorb amino acids and fat content that not fully extracted during the extraction or hydrolysis process with solvents (Sumardi, 1995).

CONCLUSIONS

1. Moringa seeds contain several beneficial nutrients such as proteins, fats, carbohydrates, vitamins E, C, B1, B2, B3, Calcium etc. The percentage of protein weight in 100 grams of Moringa seeds is significant (35.97±0.19 gr), therefore Moringa seeds have the potential to become a secondary protein source.
2. Hydrolyses can be the methods to extract protein contents from Moringa seeds. The extracted proteins can be converted into L-amino acids, nucleotides, and various peptides using solutions containing either NaOH or HCl at certain concentrations.
3. Alkaline hydrolyses produce an optimal yield of 12.1% with 0.43% protein content in the filtrate using 2% (w/v) NaOH Solution.

4. Acidic hydrolyses produce an optimal yield of 11.1% with 9.63% protein content in the filtrate using 1% (v/v) HCl Solution.
5. The different treatment of HCl and NaOH addition in the hydrolysis process will determine the type of amino acid produced

RECOMMENDATION

Protein extraction from Moringa seeds requires further studies to produce better results. These trials may involve different variations of solvent concentrations, or different solvents, or the additions of steps other than hydrolysis, or other non-hydrolytic methods all together.

ACKNOWLEDGEMENT

We would like to express gratitude to Lembaga Penelitian dan Pengabdian Masyarakat (LPPM/Research and Community Service Institute), Chemical Engineering Department, Muhammadiyah University-Jakarta for the financial support throughout this study.

REFERENCES

1. Aminah, S., Ramdhan, T. & Yanis, M., (2015). "Kandungan Nutrisi Dan Sifat Fungsional Tanaman Kelor (Moringa oleifera)". *Buletin Pertanian Perkotaan, 5*, 35-44.
2. Anggraini, A. & Yunianta, (2015). "Pengaruh Suhu dan Lama Hidrolisis Enzim Papain Terhadap Sifat Kimia, Fisika dan Organoleptik Sari Edame". *Jurnal Pangan dan Agroindustri, 3*, 1015-1025.
3. Bakhtra, D. D. A., Rusdi & Mardiah, A., (2016). "Penetapan Kadar Protein Dalam Telur Unggas Melalui Analisis Nitrogen Menggunakan Metode Kjeldahl". *Jurnal Farmasi Higea, 8(2)*, 143-150.
4. Bramantyo, A. E., Mustofa, A. & Syafril, I., (2014). "Pembuatan Bio Koagulan Dari Biji Kelor (*Moringa oleifera*) Sebagai Pengganti Koagulan Sintetis (Tawas)", Surabaya: Institut Teknologi Sepuluh Nopember.
5. Gopalakrishnan, L., Doriya, K. & Kumar, D. S., (2016). "*Moringa oleifera* : A review on nutritive importance and its medical application". *Food Science and Human Wellness, 5*, 49-56.
6. Gordon W.Selling; Mila P. Hojilla-Evangelista; Roque L. Evangelista; Terry Isbell; Neil Price; Kenneth M. Doll, (2013). "Extraction of proteins from pennycress seeds and press cake". *Industrial Crops and Products, 41*, 113-119.
7. Idris, M. A., Jami, M. S., Hammed, A. M. & Jamal, P., (2016). "*Moringa Oleifera* Seed Extract : A Review on Its Environmental Applications". *International Journal of Applied Environmental Sciences,* 11(6), 1469-1486.
8. Jubaidah, S., Nurhasnawati, H. & Wijaya, H., (2016). "Penetapan Kadar Protein Tempe Jagung (*Zea mays L.*) Dengan Kombinasi Kedelai (*Glycine maz(L.)Merill*) Secara Spektrofotometri Sinar Tampak". *Jurnal Ilmiyah Manuntung, 2(1)*, 111-119.
9. Krisnadi, A. D., (2015). "Kelor Super Nutrisi". Blora: Pusat Informasi dan Pengembangan Tanaman Kelor Indonesia.

10. Kurniawan, Lestari, S. & R.J, S. H., (2012). "Hidrolisis Protein Tinta Cumi-Cumi (*Loligo sp*) Dengan Enzim Papain". *Fishtech, 1*, 41-54.
11. Leone, A. et al., (2016). "Moringa oleifera Seeds and Oil : Characteristic and Uses for Human Health". *International Journal of Molecular Sciences, 17*, 1-14.
12. M. Purwanto, M. G., (2014). "Perbandingan Analisa Kadar Protein Terlarut Berbagai Metode Spektroskopi UV-Visible". *Jurnal Ilmiyah Sains & Teknologi, 7*, 1-71.
13. Mangale, S., Chonde, S., Jadhav, A. & Raut, P., (2012). « Study of *Moringa oleifera* (Drumstick) Seed as Natural Absorbent and Antimicrobial Agent for River Water Treatment". *Journal of Natural Product and Plant Resource, 2(1)*, 89-100.
14. Naga, W. S., Adiguna, B., Retnoningtyas, E. S., & Ayucitra, A. (2010). "Koagulasi Protein dari Ekstrak Biji Kecipir dengan Metode Pemanasan". *Jurnal Widya Teknik*.
15. Nofi, & Putra, A. N. (2013). "Pengambilan Isoleusin Dalam Konsentrat Protein Blondo Virgin Coconut Oil (VCO)". Jawa Timur: Universitas Pembangunan Nasional "Veteran" Jawa Timur
16. Nurhayati, Mappiratu & Musafira, (2018). "Pembuatan Konsentrat Protein dari Biji Kelor (*Moringa oleifera L.*) dan Analisis Profil Asam Amino". *Kovalen Jurnal Riset Kimia, 4(1)*, 24-32.
17. Wijaya, A. I., Dewi, L., & Sastrodihardjo, S. (2013). "Perbandingan Kadar Protein Terlarut Pada Berbagai Macam Tempe Dengan Variasi Bahan dari Koro Pedang (*Canavalia ensiformis L.DC*) dan Kedelai (*Glycine max (L) Merr*)". *Seminar Nasional Kimia dan Pendidikan Kimia V*, 302-308.
18. Sahay, S., Yadav, U. & Srinivasamurthy, S., (2017). "Potential of *Moringa oleifera* as a Functional Food Ingredient : A Review". *International Journal Food Science and Nutrition, 2(5)*, 31-37.
19. Salim, R. & Rahayu, I. S., (2017). "Analisis Kadar Protein Tempe Kemasan Plastik dan Daun Pisang". *Jurnal Akademi Farmasi Prayoga, 2(1)*, 19-25.
20. Sugiyono. (2004). "Kimia Pangan". Yogyakarta: Universitas Negeri Yogyakarta
21. Sumardi. (1995). "Preparasi Contoh Untuk Analisis Asam Amino Dari Berbagai Bahan Berprotein". *JKTI, 5(1)*, 46-54.
22. Triyono, A. (2010). "Mempelajari Pengaruh Penambahan Beberapa Asam pada Proses Isolasi Protein terhadap Tepung Protein Isolat Kacang Hijau (*Phaseolus radiatus L.*)". In *Seminar Rekayasa Kimia dan Proses*.1-10
23. Witono, Y., Aulanni'am, Subagio, A. & Widjanarko, S. B., 2007. Karakteristik Hidrolisat Protein Kedelai Hasil Hidrolisis Menggunakan Protease Dari Tanaman Biduri (*Calotropis gigantea*). *Berk.Penel. Hayati,* pp. 7-13.