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Amino Acid Composition of Moringa Leaf Protein

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ABSTRACT: This study aimed to investigate amino acid profile of moringa protein isolate of laboratory extracted and market sample. The extraction of laboratory protein sample was carried out using alkali-acid iso-electric precipitation method. The glutamate is most abundant amino acid followed by aspartic acid and similar trend was noticed in market samples *viz.*, glutamate and aspartic acid. The crude protein content and predicted Protein Efficiency Ratio (PER) of extracted sample- 90.26g/100g, and 1.315g/100g, market sample- 77.85g/100g and 0.40 g/100g, total amino acids of both samples 65.47, 26.24 and essential amino acids with histidine 32.76%, 37.5% and without histidine 30.97%, 36.5% respectively. Based on whole hen's egg as a reference protein, the limiting amino acids found were methionine, cystine and valine. It is best complementary supplement for combat malnutrition and shows other therapeutic effects. Difference observed in both samples may be due to varieties of moringa leaves, method of extraction, drying and storage conditions respectively.

Keywords: Moringa protein isolates, Amino acid profile, Essential amino acids.

INTRODUCTION

The world's population projected to reach 9.1 billion by 2050, approximately to 34% from present FAO (2050). Quite apart from expansion of population, steadily increasing clamour for protein is influenced by cultural developments or improvements such as increasing wealth, expanded industrialization, and geriatric populaces, where the role of protein in healthy ageing is well known, as well as greater recognition of the role of protein in a balanced diet (Henchion *et al.*, 2020). Protein is a nutrient that has been gaining popularity among consumers, with demand for plant sources of protein increasing (Hertzler *et al.*, 2020). Plant-based sources of dietary proteins dominate the supply of proteins (57%) among all available sources FAO (2010). Plant-based proteins are considered as vegan proteins since they contain substantial proportion of amino acids, are quickly absorbed by the body, and assist in the treatment of a variety of pathological disorders. Since it is costly and complex to extract an adequate amount of animal proteins, plant proteins are a feasible alternative for strengthening the human nutritional status (Schillberg *et al.*, 2019). Diet which is high in animal protein has also been linked to health issues like cardiovascular and diabetic diseases for example the replacement of 5% vegan protein for animal protein was attributed to a 23 percent reduction in the risk of type 2 diabetes (Malik *et al.*, 2016). Additionally people are extremely concerned about animal welfare, growing number of people are becoming vegetarians, vegans or flexitarians (Fischer *et al.*, 2020).

M. oleifera is a quick growing perennial angiosperm tree belongs to the family of Moringaceae. Based on different regions it is called by various names like drumstick, horse radish, kelor, marango, benzolive, maluoggay and other names. It received attention as socioeconomic importance both in torrid regions. *Moringa oleifera* consists of all edible parts such as leaf, stem, flowers and pods which are highly nutritious and considered as nutraceuticals. Because of high protein value their will be demand as supplement and in food application industries. *Oleifera* is called as multifunctional plant. In herbal medicine field it has established accelerated growth both in developed and developing countries due to its intimate source and

lowers side effects. Nearly 70% people are still adopting it as a non-allopathic medicine. From ancient times it is pre-owned as health curing agents. People found Moringa oleifera as super food to the individual suffering from hunger and deprivation. Because of its high protein quality, moringa leaves have been widely used among the medical specialists and food nutritionists to serve malnutrition and other ailments (Fahey, 2005). *M. oleifera* protein can compete effectively with protein from animal sources, significantly in relation of human body growth and enzymatic activity **(**Benhammouche, T *et al.,* 2021**).**

For the development of dietary supplements and nutritional supplements, it is critical to have an extraction process that facilitates a maximum output of vegan protein extract with high functional attributes and nutrients. The most common protein extraction method involves exposing tissue to distilled water or other weak buffers, which stipulate cell rupture and the release of intracellular proteins as a consequence of the hypotonic effect that develops over time (Maehre *et al.,* 2018). Other options entails aqueous salt or alkaline extraction, which is one of the most frequently used approaches in the laboratory for the isolation of plant-based proteins because high alkalinity aids in extracting leaf protein by breaking down hydrogen bonds, disrupting leaf tissue, and rising protein solubility (Rawdkuen 2020). The acid extraction technique is an excellent approach for isolating leaf proteins since the minimum protein solubility for *M. oleifera* leaf proteins is obtained at the isoelectric point between pH 3.2 and 4.5. When compared to concentrates extracted by heat coagulation or the addition of cationic or anionic flocculants, *M. oleifera* leaf concentrate is acidified and has the highest amino acid concentration and solubility (Santamaria-Fernández *et al.,* 2019). The objective of the study is to isolate moringa protein concentrate, production of moringa powder and evaluate the amino acid composition.

MATERIALS AND METHODS

Fresh leaves *Moringa oleifera* were collected from locally available variety farmers of Madurai.

Preparation of moringa powder. The moringa leaf powder production was carried out by using (Rawdkuen 2020) with minor modifications. Collected leaves were further processed like detaching the leaves and washed them under running water. Allowed it to dry the leaves in cabinet drier at temperature of 60°C for 72hrs until the moisture content reaches of 4%. Make the dried leaves in to fine grind powder using mixer and stored under refrigerated conditions for further analysis.

Preparation of moringa protein isolate. The moringa leaf protein isolation was carried out by using (Soo *et al.,* 2021) with minor modifications. The finely grounded fresh moringa leaf powder of 150g was mixed with 3L of water and maintained around pH of 9 by adding 1M NaOH solution. The solution was stirred for 45min at 800 RPM in a room temperature by using magnetic stirrer. Centrifuge the stirred solution at 6000 RPM for 20min at room temperature and supernatant was collected. The pH was adjusted to 4 by adding 1N HCl, allowed it to stand overnight at room temperature. The precipitated supernatant centrifuged at 6000 RPM for 10 minutes to separate precipitate from supernatant. Then collected protein precipitate was neutralized by 1M NaOH followed by freeze drying below -60° C for approximately 2.5hrs. Collected the freeze-dried protein isolate and packed in an airtight container and stored at room temperature for further analysis.

Crude protein:

Nitrogen content of moringa protein isolate was estimated by using kjeldhal method and obtained nitrogen value was multiplied with 6.25 (factor) to get crude protein value (Ma and Zuazaga, 1942).

$$\text{Nitrogen (\%)} = \frac{\text{sample titre value} - \text{blank titre value} \times 14.01 \times 0.1 \text{ (normality of HCl)}}{\text{weight of sample} \times 1000} \times 100$$

Protein (%) = Nitrogen (%) × conversion factor (6.25)

Amino acid composition: Amino acid profile of samples was determined both in and moringa oleifera leaf protein isolate and industrially processed sample by using (Kraft *et al.,* 2018). The powder samples were taken. Mobile phase A pH was adjusted with formic acid dissolving ammonium bicarbonate in 20mM water. Dissolving boric acid in water and pH was adjusted to 9.2 by using 10M NaOH, 0.2 M borate buffer was prepared. Mercaptanopropionic acid and o-phthaldialdehyde were dissolved in 10mL borate buffer solution. Prior to analysis all reagents were kept at 5°C for derivatization. Dilution of commercially available amino acid or 2.5mM of stock solution of amino butyric acid, and other amino acids in 0.1M HCl yielded 100mM standard. Sample was transferred to 1.5mL vial. Transfer of 45µL of 0.2M borate buffer. Mixing and allow it to stay for 1minute and inject 1µL of mixture was injected for HPLC analysis.

Predicted protein efficiency ratio: PER was calculated according to (Alsmeyer *et al.,* 1974) by using the given formula

P-PER = -0.468+0.454(Leu)-0.105 (Tyr)

RESULTS AND DISCUSSION

The crude protein content was analysed in commercially available moringa protein and from extracted moringa protein. The results demonstrated that extracted protein isolate from moringa exhibits higher amount of crude protein *viz.,* 90.26% and commercially available protein is about 77.85 respectively. The results of isolated moringa leaf protein at laboratory level show similar trend with rice protein isolate studied by (Zhang *et al.,* 2018). The variation between the samples due to the effect of some crucial factors *viz*., extraction method, temperature, pH, drying techniques and other. The process of soaking for six to eight hours may increase the retention of protein,

when freeze drying prolongs the protein get denaturation due to raise of temperature. In comparison with spray drying and freeze drying, freeze drying is better to retain crude protein without loss of structure and some functional properties of protein. When protein sample were spray dried and subjected to heat at 180°C there may be some denaturation. Protein content significantly depends up on the drying of leaf according to (Soo *et al.,* 2021). There may be significant difference in amino acid composition also.

The amino acid compositions of both samples were mentioned in Table 1&2. In this study the amino acid concentration differed in samples due to extraction, production environment and agricultural practices. The moringa protein isolate was high in glutamate of 13.79 (g/100g) among the all amino acids, second position is occupied by acidic amino acid aspartic acid of 9.44 (g/100g). cystine and methionine are the least concentrated amino acids of 0.63 (g/100g) and 0.19 (g/100g) respectively. In industrially processed moringa protein isolate we can notice the same trend but the concentration may vary viz., glutamate 6.221 (g/100g), aspartic acid 4.108 (g/100g). According to (Adeyeye *et al.,* 2010) noticed that difference in lysine, histidine, arginine and cystine were severely impaired when protein is subjected to heat, improper processing and storage. By considering the point stated by (Adeyeye *et al.,* 2010) the similar observations was noticed in this study. The amino acid concentration was noticed more in freeze dried sample compared to spray dried commercial sample (Industrially moringa protein). The total hydrophilic and hydrophobic amino acid composition was significantly higher in laboratory freeze dried moringa protein compare to industrially processed protein. The same was noticed by (Branchs *et al.,* 2017) in the study of soya protein isolates. The (Hayeti *et al.,* 2019) stated that glutamic acid concentration will be high due to maintenance of isoelectric pH during extraction process the similar trend was also noticed in grass protein isolate. (Branchs *et al.,* 2017) stated that amino acid concentration decides the behaviour of protein. According to (Nakai, and Modler 1996) the proportion of hydrophobic and hydrophilic amino acids has impact on solubility, water binding ability and surface characteristics. The number of hydrophilic groups in the protein isolate increases the solubility. Even though freeze dried protein isolate is rich in amino acid composition compare to industrially moringa protein (IMP).

Table 1: Amino acid profile of moringa protein isolate (g/100g) of crude protein.

Sr. No.	Amino acid	MPI	Mean
1.	Histidine	1.17±0.02	1.2
2.	Lysine	2.38±0.06	2.4
3.	Leucine	4.25±0.07	4.3
4.	Isoleucine	2.45±0.02	2.5
5.	Methionine	0.193±0.002	0.19
6.	Phenylalanine	5.28±0.08	5.2
7.	Threonine	2.77±0.05	2.78
8.	Valine	2.96±0.05	2.96
9.	Aspartic acid	9.44±0.26	9.4
10.	Glutamate	13.79±0.27	13.8
11.	Serine	3.60±0.03	3.6
12.	Glycine	2.01±0.02	2.0
13.	Arginine	4.96±0.10	4.97
14.	Alanine	6.03±0.008	6.03
15.	Tyrosine	1.31±0.02	1.31
16.	Cystine	0.63±0.01	0.63
17.	Proline	2.25±0.02	2.3

Table 2 Amino acid profile of industrially processed moringa protein isolate g/100g of crude protein.

Sr. No	Amino acid	IMP	Mean
1.	Histidine	0.285±0.004	0.3
2.	Lysine	1.00±0.01	1.0
3.	Leucine	1.98±0.02	2.0
4.	Isoleucine	1.097±0.003	1.1
5.	Methionine	0.01±00	0.0
6.	Phenylalanine	2.45±0.01	1.45
7.	Threonine	1.50±0.04	1.50
8.	Valine	1.55±0.01	1.55
9.	Aspartic acid	4.108±0.09	4.11
10.	Glutamate	6.221±0.19	6.2
11.	Serine	1.331±0.04	1.3
12.	Glycine	0.010	0.01
13.	Arginine	1.683±0.03	1.68
14.	Alanine	2.238±0.04	2.2
15.	Tyrosine	0.203±0.004	0.2
16.	Cystine	0.030	0.0
17.	Proline	0.55±0.002	0.6

Young and Pellett (1994) noticed that both have balanced amino acid content so it is useful for human consumption. Stadtlander and Becker (2017) revealed that the major non-essential amino acid in moringa leaves were glutamic acid, aspartic acid, arginine, proline, glycine and also stated that the crude protein content ranged between 188g/Kg to 277g/Kg in different varieties and species of moringa.

Each amino acids plays crucial and important role in human life *viz.*, valine- stimulates the growth and regeneration of muscle, threonine- fat metabolism, immune function, component of skin and connective tissue, Leucine- regulate blood sugar levels and growth hormones, isoleucine- plays role in haemoglobin production and energy regulation, lysine- protein synthesis, calcium absorption, hormone and enzyme production, histidine- digestion and sleep awake cycle respectively.

Table 3: Concentration of non-essential, essential, acidic, neutral, sulphur, aromatic of MPI and IMP (g/100g crude protein).

Amino acid composition	MPI	IMP
Total amino acids	65.47	26.24
Total essential amino acids		
Without histidine	20.28	9.58
With histidine	21.45	9.86
Total non-essential amino acids	44.02	16.35
% of total essential amino acids		
Without histidine	30.97	36.5
With histidine	32.76	37.5
% of total non essential amino acids	67.23	64.77
Total acidic amino acids	23.23	10.32
Total basic amino acids	8.51	2.968
Total neutral amino acids	33.73	12.948
Total sulphur amino acid	0.82	0.04
Total aromatic amino acids	6.59	2.653
% Total acidic amino acids	35.48	38.98
% Total basic amino acids	12.99	11.28
% Total neutral amino acids	51.5	49.3
% Total sulphur amino acid	1.252	0.15
% Total aromatic amino acids	10.06	10.09

Abundant parameters are mentioned in Table 3. The total amino acids composition of both samples were 65.47g/100g and 26.24g/100g highest total amino acids (TAA) was noticed in moringa protein isolate (MPI) samples which was according with (Benhammouche *et al.,* 2021) of value were found to be around 89.8mg/g. The percentage of total essential amino acids with histidine and without histidine of both samples was ranged between 30 - 37%, percentage of total non-essential amino acids of both samples were ranged between 64-67% respectively. The results were somewhat familiar with (Rawdkuen 2020). Most of our results were better in many of the essential amino acids compare to pumpkin (Olaofe *et al.,* 1994). According to the results obtained in both samples the amino acids values are little higher or within range according to (FAO/WHO 2007). The aromatic amino acids in MPI was 10.06 % and IMPI 10.09 % the obtained results were in contrast with (Moyo *et al.,* 2011), sulphur containing amino acids were 1.25% and 0.15% were in similar trend with (Alain Mune Mune M *et al.,* 2016). The lysine, valine threonine are high in both samples which were in contrast with (Olaofe *et al.,* 2013) While it is recognised that cystine can provide a portion of methionine requirement, WHO/FAO/UNICEF (1985) does not specify the proportion of total sulphur containing amino acids that cystine can meet. Most animal protein are low in cystine, howerever many vegetable proteins have far cystine and methionine. As a result, Cystine is unlikely to account for more than half of the total sulphur amino acids in animal protein (FAO/WHO, 1991). The predicted protein efficiency ratio (P-PER) of both the samples were 1.315 for moringa protein isolate (MPI) and 0.40 for industrial moringa protein (IMP) respectively. According to (Lalitha *et al.,* 2020) the PER value was ranged between (2.8 to 3.3).

Amino acid score based on provisional amino acids were mentioned in table-5 limiting amino acid content of both samples were methionine + cystine with values of 0.23 and 0.01 respectively. There is no significant difference between MPI and IMP samples. According to (Adeyeye *et al.,* 2010) in order to fulfil the requirement of sole protein diet 2.3 or 1.96 times more consumption in needed.

Table 4: The crude and predicted protein values of both MPI and IMP (g/100g).

Parameters	MPI	IMP
Crude protein content	90.26	77.85
Predicted Protein efficiency ratio	1.315	0.40

Table 5: Essential amino acid score of MPI and IMP based on provisional amino acid score.

Amino acid	MPI	IMP
Leucine	0.60	0.28
Lysine	0.36	0.15
Valine	0.49	0.25
Threonine	0.69	0.37
Isoleucine	0.61	0.27
Methionine+cystine	0.23	0.01
Phenylalanine+tyrosine	1.09	0.44

Table 6: Essential amino acids score of MPI and IMP based on whole hens egg.

Amino acid	MPI	IMP
Histidine	0.48	0.11
Lysine	0.36	0.15
Leucine	0.46	0.21
Isoleucine	0.37	0.16
Methionine	0.03	0.001
Phenylalanine	0.52	0.24
Threonine	0.55	0.3
Valine	0.4	0.20
Tyrosine	0.12	0.02
Cystine	0.11	0.005

Table 6 contains essential amino acid score based on whole hens egg. Methionine, cystine, valine are the limiting amino acids in MPI and IMP respectively. The following results of EAA, TAA, TNEAA, EAA score following results were obtained with significant difference 0f $P < 0.5$.

CONCLUSION

The findings of this study were concluded that amino acid concentration was seen more in MPI sample compare to IMP sample this may be due to series of reaction occurs in the extraction and drying time. Also difference observed in both samples may be due to varieties of plants from where it procured method of extraction, drying and storage conditions respectively. Due to increase in preference of plant protein by most of population both protein samples have balanced proteins according to FAO so individual amino acid has its role in human body so suggestion is to moringa consume protein at least one time in a day.

FUTURE SCOPE

Moringa protein isolate can be used in development of novel food products such as protein mix in combination, beverages, candies, bars and other foods may be used as nutritional treatment strategies. The protein or incorporated products are available to consumers at reasonable price and it will be profitable for both producers, farmers. The protein isolates incorporated products can prevent malnutrition and other problems of humans which make available throughout the year respectively.

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Conflict of Interest. None.

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