ISOLATION AND AMINO ACID PATTERN ANALYSIS OF WATER SOLUBLE PROTEIN FROM TURMERIC OF BANGLADESHI ORIGIN AND DETERMINATION OF ITS PEPsin DIGESTIBILITY

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ABSTRACT

The present study was designed to isolate water soluble protein from turmeric of Bangladeshi origin and analysis of its amino acid pattern. The study also determined the pepsin digestibility of the isolated protein. Proximate composition analysis is a main prerequisite of the current study to find the protein content of turmeric. Proximate composition analysis was performed using AOAC method. An effective low cost method was applied for the extraction of water soluble protein from turmeric. Amio acid profile of the isolated protein was determined using the method described by Bao Yang et al.. Pepsin digestibility is calculated on the basis of AOAC Official method 971.05. The result of proximate composition analysis showed that turmeric contains a significant amount of protein (9.45%), moisture, ash, fat, fiber and carbohydrate. The maximum concentration of the protein isolate was found significant (85.34%) at pH 1.23. Besides, Pepsin digestibility of the isolated protein was found 96.13%. Analysis o amino acid profile determined the presence of 14 amino acids where 8 are essential and 6 are non-essential amino acid.

KEYWORDS: Turmeric, Curcuma longa, Protein, Amino acid, Pepsin digestibility.
INTRODUCTION

Nature is a great source of salvation for human being by providing different remedies from its plants, animals and other sources to treat all ailments of mankind. Among all of the natural sources, medicinal plants are important contributors. Medicinal plants have a long history of serving people in many regions of the world. The use of medicinal plants for the treatment of many diseases is associated to folk medicine. Natural products from some plants, fungi, bacteria and other organisms, continue to be used in pharmaceutical preparations either as pure compounds or as extracts. Report shows that almost 80% of the world population still uses medicinal plants to maintain their health and to cure their ailments. Medicinal plants and herbs always play an important role in the development of health in mankind. Man has been using herbs and plant products for combating diseases since times immemorial. It is now established and fully believed that phytoconstituents obtained from the medicinal plants serve as pilot molecules in the modern medicines and many people still depend on the traditional medicine for their preliminary health care and treatment. The Indian subcontinent is enriched by a variety of flora- both aromatic and medicinal plants. This extensive flora has been greatly utilised as a source of many drugs in the Indian traditional system of medicine. Turmeric or Curcuma longa, is one such medicinal plant explained extensively in Indian material medica (Dravyaguna Sastra). Botanically it is related to Zingiberaceae family. Curcuma longa (C. longa) is widely used as a spice and colouring agent, and is well known for its medicinal properties. Actually, C. longa, or turmeric is a perennial herb and is cultivated extensively in Asia. Generally, the rhizome of C. longa is used medicinally. Dried rhizome is the source of turmeric, which gives curry powder by yielding its characteristic yellow color. Rhizome is useful in the treatment of diabetics, hemorrhoids, anemia, jaundice, cough, asthma, wound healing, colic, gout, renal calculi, poisoning, freckles, skin and neurological disorders. Tribal women of Assam apply paste of fresh rhizome on the skin to protect it from infection and enhance the complexion. Rhizome of this plant along with other ingredients is given to cattle to treat loose stools. Oral administration is the main route of administration for C. longa, it can also be used topically for the treatment of acne, boils, bruises, blistering, ulcers, eczema, insect bites, parasitic infections, hemorrhages and skin diseases like herpes zoster and pemphigus. The active constituents of turmeric are the flavonoid Curcuminoids which is a mixture of curcumin (diferuloylmethane), monodexmethoxycurcumin and bidesmethoxycurcumin. Curcumin makes up approximately 90% of the curcuminoid content in turmeric. Other constituents include volatile oils, sugars, proteins, and resins. Curcumin is the best
researched active constituent which is responsible for the biological activities of turmeric. Curcumin, a potent antioxidant is believed to be the most bioactive and soothing portion of the herb turmeric and possess the properties like antioxidant, anti-inflammatory, anti-platelet, cholesterol-lowering antibacterial and anti-fungal effects. It contains a mixture of powerful antioxidant phytonutrients known as curcuminoids. It is a strong anti-oxidant, which supports colon health, exerts neuroprotective activity and helps to maintain a healthy cardiovascular system.

The main purpose of our present study was to isolate and amino acid pattern analysis of water soluble protein from turmeric of Bangladeshi origin. We also determined the proximate composition of turmeric and pepsin digestibility of its isolated protein.

MATERIALS AND METHODS

Sample Preparation: Raw turmeric (Curcuma longa) was purchased from a local market (New market) in Dhaka, Bangladesh. Fresh raw turmeric was peeled and washed in running tap water to remove adhering debris, sliced into chips and dried in sun for 4 days. Dried chips were ground into fine powder by using a commercial blender (Jaipan, IS: 4250). The powdered samples were stored into an air tight bottle in freeze (about 4°C) until further analysis.

Chemicals
Sulfuric Acid, Boric Acid, Digestion mixture (3 kg K₂SO₄ and 600 g CuSO₄), Hydrochloric Acid, Sodium Hydroxide, Petroleum Ether, Acetone, Ascorbic Acid Std. Solution, 2,6-Dichloroindophenol Std. Solution, Metaphosphoric Acid, Acitic Acid, Silver Nitrate Solution, and Pepsin are used in the current study. All of these chemicals are of analytical grade.

Determination of proximate composition of turmeric

Moisture content: The moisture content of powdered turmeric sample was determined by using AOAC method 934.01 as mentioned by EK Ileleji et al. in this method loss on drying is measured by drying the sample in an oven at 105 °C for 5 hours.

Protein Content
The powdered turmeric sample was tested for crude protein content according to the Kjeldahl’s method as reported by JM Lynch et al. which involved protein digestion and
distillation. In digestion stage, 2.0 g turmeric powder was digested by 15-20 ml of 98% Sulfuric acid in a 250 ml Kjeldhal flask, 1 g of digestion mixture added to act as catalyst. The resulting mixture was subjected to heat in the digestion chamber until it become transparent followed by normal cooling. The cooled digest was transferred into a 100 ml volumetric flask and made up to the mark with distilled water. In a Markham distillation apparatus 5.0 ml digest was taken via a small funnel aperture. This funnel and the inner surface of the apparatus were washed with sufficient amount of distilled water 66 followed by the addition of 3-4 drops of phenolphthalein and 5 ml of 40 % (W/V) NaOH solution. In a 100 ml conical flask 5 ml of 2 % boric acid and 1 or 2 drops of mixed indicator was taken and placed under the condenser such that the condenser tip was under the liquid. The digest in the condenser was steamed through until enough ammonium sulfate was collected. The Boric acid with indicator solution changed color from red to green showing that all the ammonia liberated had been trapped. The solution in the receiving flask was titrated against 0.063 N hydrochloric acid till the appearance of purple color.

**Crude Fat**

The crude fat in the powdered sample was determined using Soxhlet extraction method (AOAC Official Method 920.39) as mentioned by NJ Thiex et al.\(^{[13]}\) In this extraction method fat is extracted with petroleum ether. Samples (3.0 g) weighed accurately into labeled thimble and 150 ml of petroleum ether (boiling point 40-60°C) was taken in a 250 ml boiling flasks. The extraction thimbles were plugged tightly with cotton wool. After that, the Soxhlet apparatus was assembled and allowed to reflux for 24 hrs. The thimble was removed with care and petroleum ether collected from the top container and drained into another container for re-use. After that, the boiling flask was heated in a hot air oven until it was almost free of petroleum ether. After drying, it was cooled in desiccators and weighed.

**Fiber**

Crude Fiber content is determined using the method described by M Jrgen.\(^{[14]}\) based on AOAC Official Method 978.10 with some necessary modification, 2 g fat free sample of powdered turmeric was taken into a fiber flask and reflux with 100 ml of 0.255 N H\(_2\)SO\(_4\) for one hour. After filtration, the difference obtained was thrown off and the residue was refluxed with 100ml of 0.313 M NaOH for one hour. The mixture was filtered through a fiber sieve cloth and washed with 10 ml of acetone to dissolve any organic constituent followed by washing with 50 ml of hot water for twice. The resulting residue was oven dried at 1050°C.
overnight to drive off moisture. Then it is subjected to cooling in a dessicators and weighed (W1) for ashing at 5500 °C for 4 hours. The resulting white and grey ash (free of carbonaceous material) was cooled in desiccators and weighted to obtain W2.

The % of crude fiber was calculated as follows-

\[
\text{Fiber (\%) = \{[(W1 – W2) / Wt. of sample] \times 100\}
\]

**Ash**

Ash is an inorganic residue remaining after the material has been completely burnt at a high temperature. It is the aggregate of all non-volatile inorganic elements. According to AOAC Official Method 942.05 used by NJ Thiex et al. \[15\] samples are burnt at 600°C for 2 hours. 8 g of finely ground dried sample was weighed into a porcelain crucible. Placed the crucible in a muffle furnace and heated at 6000 C for 2 hrs. The ash was cooled in desiccators and reweighed to calculate the amount of ash obtained.

**Total Carbohydrate**

The total percentage carbohydrate content in the dried turmeric powder sample was determined by the difference method. This method involved adding the total values of crude protein, lipid, crude fiber, moisture and ash constituents of the sample and subtracting it from 100 \[16\] the value obtained is the percentage carbohydrate constituent of the sample.

Thus, % carbohydrate = 100 – (% moisture + % crude fiber + % protein + % lipid + % ash)

Energy value: The energy value of the samples was determined by multiplying the protein content by 4, carbohydrate content by 4 and fat content by 9 and then the summation will give the energy value. \[17\]

\[
\text{Energy Value} = (\text{Crude protein} \times 4) + (\text{Total carbohydrate} \times 4) + (\text{Crude fat} \times 9)
\]

**Isolation of water soluble protein**

This assay was determined by the help of the method reported by M Chethankumar, \[18\] with several modifications as required. Powered samples were defatted by treating it with petroleum ether solvent in a Sohxlet apparatus for 24 hours. The defatted samples were dried at room temperature in a fume hood. 5 g of the defatted sample is than treated with warm double distilled water. The resulting suspension was mixed thoroughly using a magnetic stirrer for 15min at room temperature and allowed to stand overnight at 4°C to extract the protein with water. The solution was filtered first by sieve cloth and then through Whatman
no.1 filters paper. The liquid was kept into a receiving flask. The separated solids were then washed twice with distilled water each time decanting through the filter paper into the same receiving flask. The resultant suspension was centrifuged at 10,000 rpm, 20min, at 4°C. The supernatant was filtered through Whatmann No.1 filter paper and Sartorius filter (0.045μm) and kept at 4°C.

Protein was precipitated from supernatant solution by the principal of isoelectric precipitation. [19] 0.04608N HCl is added dropwise to 20 ml supernatant liquid in conical flaks. First precipitation appears after reaching pH 4.1and continued to increase till pH 1.23 and at 1.23 maximum precipitations is found. This precipitation is than separated through centrifugation (10,000 rpm), for 15 min in a centrifuge machine. The protein was then washed with water, dried by freeze drying and stored at 5°C for further analysis.

**Amino acid pattern of isolated protein**

Amino acid profile of the isolated protein is determine by the help of the method described by Bao Yang et al. [20] with some modifications. Freeze dried isolated protein sample was taken and a fine paste was made by mortar and pestle using 6N HCl and filtered through whatman no. 1 filter paper and take it in 250 mL round bottom flask. The flask is then placed in a heating mantle at 110°C for 22 hours for hydrolysis of protein. After hydrolysis, the solution was kept in a evaporating dish to evorapate HCl on water bath. It was then filtered through Whatman no. 1 filter paper in a 25 mL volumetric flask with 0.1 N HCl. The 25 mL solution was kept as Stock solution. Again the stock solution filtered through 0.45 μm syringe filter. Then the stock solution and standard solution were run through the amino acid analyzer. The analyzer showed the standard curve for standard solution and another curve for stock (sample) solution. By comparing the two curves, the amount of amino acids were calculated.

**Pepsin Digestibility of isolated Protein**

Pepsin Digestibility is calculated on the basis of AOAC Official method 971.05 as described by Eric L. Miller et al. [21] In a 700 ml round bottom flask 2 gm moisture free sample was taken followed by the addition of 490 ml distilled water, 1.5 gm pepsin and 10 ml 25% HCl respectively in the flask. The flask with the resulting solutions was put in an incubator at 380 C temperatures for 24 hours. After completion of 24 hours, 10 ml 25% HCl was added in the flask and kept in the incubator at 380C for 6 hours. The insoluble residue was separated by filtering with ash less filter paper, washed by hot water and dried in normal temperature.
Then the ash less filter paper weighted with residue to estimate the protein according to the Kjeldahl’s method.

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Calculation

\[
\text{Protein digestibility} = \left( \frac{\% \text{ total protein content} - \% \text{ protein after digestion}}{\% \text{ total protein content}} \right) \times 100.
\]

RESULTS AND DISCUSSION

Proximate composition analysis of turmeric

In the current investigation proximate analysis is carried out to find out protein, moisture, ash, fat, crude fiber, and carbohydrate content in the turmeric. The result obtained is tabulated in the following table (Table-1).

Table-1: Proximate composition of turmeric (Curcuma longa) of Bangladeshi Origin.

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Crude Protein</td>
<td>9.45 ± 0.50%</td>
</tr>
<tr>
<td>2.</td>
<td>Moisture</td>
<td>10.70 ± 0.30%</td>
</tr>
<tr>
<td>3.</td>
<td>Ash Content</td>
<td>7.71 ± 0.04%</td>
</tr>
<tr>
<td>4.</td>
<td>Fat Content</td>
<td>3.56 ± 0.20%</td>
</tr>
<tr>
<td>5.</td>
<td>Crude Fibre</td>
<td>5.92 ± 0.60%</td>
</tr>
<tr>
<td>6.</td>
<td>Carbohydrate</td>
<td>62.66 ± 0.60%</td>
</tr>
</tbody>
</table>

Testing sample (dried turmeric powder) contains a significant amount of protein, moisture, ash, fat, fiber and carbohydrate. The testing sample contains 9.45% protein. The amount of protein in the testing sample is quite significant. In general turmeric contains 6-8% protein.[22]
that is comparable with the test result found from Turmeric of Bangladeshi origin. It is thus evident that the turmeric of the Bangladeshi origin can be a good source of Protein Supplement. Due to the deficiency of protein all over the world alternative sources for protein should be find out. In this consequence Protein in Turmeric can be a good alternative, if it is sufficiently digestible in pepsin. \[^{22}\] Turmeric of Bangladeshi origin may contain a distinctive amount of protein than turmeric from other origin.

![Composition of Turmeric Rhizome of Bangladeshi Origin](image)

**Figure-1: Poroximate composition of turmeric of Bangladeshi Origin.**

**Water soluble protein isolation from turmeric**

Turmeric used in the present study bears 9.45% crude protein by weight. Protein after extracted with water is isolated by using isoelectric point precipitation. Usually the isoelectric points for plant protein are about pH 4.5 or less. \[^{23}\] A suitable isoelectric point for total protein in turmeric is needed to find out to maximize the precipitation.

**Table-2: Protein isolation at different Ph.**

<table>
<thead>
<tr>
<th>S. No</th>
<th>PH</th>
<th>Protein Isolated, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.10</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>3.40</td>
<td>16.66</td>
</tr>
<tr>
<td>3</td>
<td>2.43</td>
<td>59.11</td>
</tr>
<tr>
<td>4</td>
<td>1.23</td>
<td>85.34</td>
</tr>
</tbody>
</table>

Result shows that protein precipitation increases with the lowering of pH of the resulting solution. It is also noticeable that protein precipitation get started at pH 4.10 and it get
maximize at pH 1.23 in which 85.34% protein is precipitated. As a result pH 1.23 is taken as a suitable pH for the isolation of total protein from turmeric in this study.

![Protein isolated at Different PH](image)

**Figure-2: Protein precipitation with the change of pH**

**Amino acid pattern of isolated protein**

Freeze dried isolated protein is tested for amino acid pattern to prepare an amino acid profile. In the current study the analyzer showed two curves, one for standard and another for the current sample, by comparing the peaks in these two curves the amount of amino acid in the resulting protein is estimated. In the present analysis, the isolated protein contains 14 amino acids among 20 amino acids that are important for human. Result shows that resulting protein contain 8 essentail amino acid and 6 Non-essentail amino acid. In the Isolated protein the essential amino acid Lysine (12.73%) is the maximum in content and Valine (1.53%) is in Minimum. On the other hand among the Non-Essential Amino Acids Glutamic Acid (8.75%) is maximum and Serine (2.29%) is found minimum.

**Table-3: Essential and Non-Essential Amino Acid found in the isolated protein.**

<table>
<thead>
<tr>
<th>Essential Amino Acid</th>
<th>Content (%)</th>
<th>Non-Essential Amino Acid</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>2.48</td>
<td>Alanine</td>
<td>2.55</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.80</td>
<td>Aspartic acid</td>
<td>5.05</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>7.58</td>
<td>Glutamic acid</td>
<td>8.75</td>
</tr>
<tr>
<td>Leucine</td>
<td>2.53</td>
<td>Glycine</td>
<td>3.42</td>
</tr>
<tr>
<td>Lysine</td>
<td>12.73</td>
<td>Serine</td>
<td>2.29</td>
</tr>
<tr>
<td>Methionine</td>
<td>3.28</td>
<td>Tyrosine</td>
<td>3.68</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>1.53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Pepsin Digestibility of isolated Protein

Water soluble protein has been isolated from the turmeric powder at pH 1.23. The precipitation obtained at pH 1.23 contains 85.34% protein. This protein is tested for pepsin digestibility. Pepsin is an enzyme secreted from stomach. It works on protein, assists protein digestion by breaking peptide bonds. \(^{24}\) Pepsin digestibility of the isolated protein in our study is 96.13%. This indicates the high purity of the isolated protein and this may be taken as a food in daily intake.

CONCLUSION

Turmeric has a long history of use, not just as a spice, but also as a healing agent and as a magical herb. Turmeric has been used in South India for thousands of years and is a major part of Siddha medicine. \(^{25}\) Here, based on the results of our study, we may conclude that turmeric of Bangladeshi origin contains a great deal of protein. The resulted protein is 96.13% digestable in pepsin. Our study also revealed that turmeric possess many useful phytoconstituents which are responsible for its efficacy. Hence, it can be considered a great potential therapeutic agent.

CONFLICTS OF INTEREST

All authors have none to declare

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