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AMINO ACIDS COMPOSITION AND PEPSIN DIGESTIBILITY OF PROTEIN ISOLATED FROM TURMERIC *(CURCUMA LONGA* L.*)* PRODUCED IN BANGLADESH

Rajib Chandra Das[1], Ashish Kumar Sarker*[2], Asaduz Zaman[3] and Muhammed Yousuf Miah[1]

[1]Department of Applied Chemistry and Chemical Engineering, Noakhali Science and Technology University, Noakhali-3814, Bangladesh.

[2]Plant Protein Research Section, Institute of Food Science and Technology, Bangladesh Council of Scientific and Industrial Research, Dhaka-1205, Bangladesh.

[3]Department of Applied Chemistry and Chemical Engineering, University of Dhaka, Dhaka-1000, Bangladesh.

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***Correspondence for Author**
Dr. Ashish Kumar Sarker
Plant Protein Research Section, Institute of Food Science and Technology, Bangladesh Council of Scientific and Industrial Research, Dhaka-1205, Bangladesh.
ashish10608@hotmail.com

ABSTRACT

Turmeric (rhizome of the herb *Curcuma longa* L., Zingiberaceae) protein has been focused for its antioxidant and anticancer agents. The present study was designed to isolate water soluble protein from turmeric produced in Bangladesh and analysed for its amino acids pattern. The result of proximate composition analysis showed that turmeric contains 9.45% protein. It was obtained by ethanol precipitation following the extraction of protein at isoelectric *p*H (1.23) and the maximum concentration of the protein in protein isolate was found 85.34%. The amino-acids patterns of these isolated proteins were analyzed and found that essential amino acids such arginine (2.48%), histidine (1.80%), isoleucine (7.58%), leucine (2.53%), lysine (12.73%), methionine (3.28%), threonine (2.87%), valine (1.53%) and non essential amino acids such as alanine (2.55%), aspartic acid (5.05%), glutamic acid (8.75%), glycine (3.42%), serine (2.29%) and tyrosine (3.68%) were present in turmeric protein. Pepsin digestibility of the isolated protein was found 96.13%.

KEYWORDS: Turmeric, Protein isolation, Amino acid and Pepsin digestibility.

INTRODUCTION

Turmeric or *Curcuma longa*, is one kind of medicinal rhizome explained extensively in Indian material medica (Dravyaguna Sastra).[1] Botanically it is related to Zingiberaceae family.[2] It is widely cultivated in Asia, used as a spice and colouring agent, and is well known for its medicinal properties.[3] Dried rhizome is the source of turmeric which becomes a special importance for human because of its pleasant yellow color and aroma.[4] It is useful in the treatment of diabetics, hemorrhoids, anemia, jaundice, cough, asthma, wound healing, colic, gout, renal calculi, poisoning, freckles, skin and neurological disorders.[5] 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.[1] 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.[4] Curcumin makes up approximately 90% of the curcuminoid content and the remaining 10% are other constituents such as moisture, volatile oils, sugars, proteins, and resins.[6] Turmeric extracts have been reported to have effect as antimicrobial, anti inflammatory, antioxidant and anticancer agents.[7] The significance of turmeric in medicine has changed considerably since the discovery of the antioxidant properties of naturally occurring phenolic compounds. Mukunda Chethankumar *et al*. used turmeric peel waste to successfully develop a process that produced antioxidant protein.[8]

Turmeric proteins have good medicinal properties and could be incorporated in food, but their functional properties have not been extensively determined to utilize these valuable sources of protein for food preparation. More information about its amino acids concentration and pepsin digestibility is needed. The aim of the present study was to isolate the water soluble antioxidant protein and determined its amino acid pattern that will help the manufacturer for producing protein concentrate or isolate from turmeric having good medicinal properties.

MATERIALS AND METHODS

Materials

Bangladeshi origin raw turmeric (*Curcuma longa*) was collected from local market. Analytical grade sulfuric acid, boric acid, potassium sulphate, copper sulphate, hydrochloric acid, sodium hydroxide, petroleum ether, acetone, 2,6-dichloroindophenol, metaphosphoric acid, acetic acid, silver nitrate, ascorbic acid standard solution and pepsin were used without further purification.

Methods

Sample Preparation

Fresh raw turmeric was peeled and washed in running tap water to remove adhering debris, sliced into chips and dried in sun for 7 days. Dried chips were ground into fine powder by using blender (Jaipan, IS: 4250) and powder were stored in freeze for further analysis.

Proximate Analysis of Turmeric

The nutritional compositions of samples such as moisture, minerals, crude fibre and fat were determined in the laboratory using the standard AOAC method 950.46, AOAC method 920.153, AOAC method 985.29 and AOAC method 960.39 respectively. [9] Crude protein was determined by the micro Kjeldahl method and reported as %N x 6.25.[10] The total carbohydrate was estimated by subtraction of moisture, crude fiber, crude protein, and fat percentage from 100. The energy value of the samples was determined by the multiplication of protein and carbohydrate content with 4 and fat content with 9.

Protein Isolation

The protein was isolated by the modified method of Chethankumar (2010).[11] The protein extractability was determined by contacting 20 g of ground defatted turmeric powder with warm double distilled water. The resulting suspension was mixed thoroughly by using a magnetic stirrer for 1 h and allowed to stand overnight at 40 ºC. The extract and solids were separated by centrifugation at 12000 rpm. The liquid was decanted and vacuum filtered through Whatman 41 paper to a receiving flask. The solids were washed twice with distilled water and each time decanting through the filter paper into the same receiving flask. The soluble protein was precipitated by adding HCl at the pH range of 4 to 1. The extractability was measured as the mass ratio of the recovered protein in the collected extract solution compared with that in the 20 g of starting material. This precipitated protein was than separated through centrifugation (10,000 rpm), for 15 min in a centrifuge machine, dried and stored for further analysis.

Amino Acid Pattern of Isolated Protein

Amino acid composition of protein isolates was determined by using an amino acid analyzer (Shimadzu, Japan) and only fourteen amino acids were determined due to the limitation of the instrument. 0.5 g isolated protein was pasted with 50 ml 6 N HCl by mortar pestle and then filtered by Whatman 41 filter paper. The filtrate was placed in a hydrolysis tube and hydrolyzed for 22-24 hours. After hydrolyzing, HCl of the filtrate was evaporated in water

bath and added three times distilled water in it. The filtrate re-evaporated until complete evaporation of HCl occurred. After evaporation, about 10ml solution was taken and made volume by 0.1 N HCl in 25 ml volumetric flask. The stock solution was used for amino acids analysis.

Pepsin Digestibility

The in vitro protein digestibility was carried out according to the modified method of Mertz et al. (1984).[12] 2.0 g moisture free sample was taken in 1000 ml round bottom flask. A mixture of 490 ml distilled water and 1.5 g pepsin was added in it. Then 10 ml 25% HCl was added and the solution mixture kept in incubator at 37^0C for 24 hours with occasional stirring. After this treatment, 10 ml of 25% HCl was added in the mixture again and kept in an incubator at 37^0C for 6 hours. After incubation the reaction was stopped by addition of 15 ml of 10% trichloroacetic acid. The mixture was then filtered quantitatively through Whatman No. 1 filter paper. Supernatants were discarded and pellets were washed with purified distilled water. Pellets were saved for protein determination. Undigested nitrogen (N) was determined by micro-Kjeldahl method. Digestibility was calculated as:

% Digestibility = (N in sample – undigested N)/N in sample × 100.

Statistical Analysis

Three replicates were carried out in each experiment. All data were analyzed by SPSS software, version 15 using one-way ANOVA analysis. The level of statistical significance was set at 5% ($p<0.05$).

RESULTS AND DISCUSSION

Proximate Analysis of Turmeric

Proximate chemical compositions of turmeric powder estimated are presented in Fig. 1. Theturmeric powder contains high crude protein of 9.45 ± 0.50% hence appeared to be a moderately good source of protein. The protein content in turmeric were reported 6-8% that our investigation results are comparable with literature.[13] On the other hand, ether extract content (3.56 ± 0.20%) was low compared with other nutrients. Crude fibers content (5.92 ± 0.60%) was found higher value that hindered the digestibility of protein. Total minerals content was found 7.71 ± 0.04% to be indicating that it is a good source of minerals. The vitamin C (ascorbic content) in turmeric powder was found 27.32 mg/100 g which also assigned the good source of vitamin C.

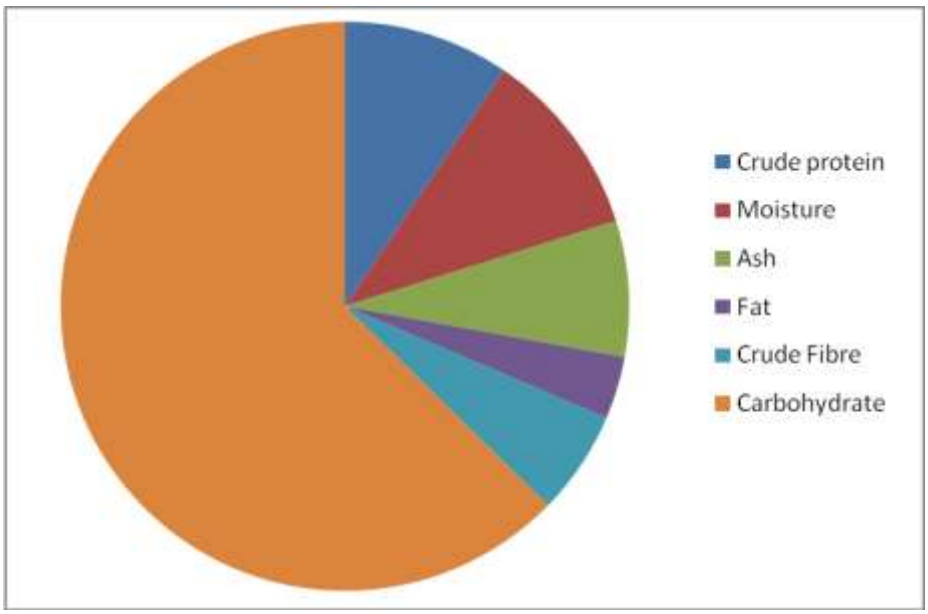

Fig 1: Proximate composition of turmeric powder.

Protein Extraction

Protein extraction of turmeric is normally governed by the extraction time and warm condition and then precipitation at isoelectric point by lowering pH. The data of the study indicates that water soluble protein precipitation was gradually decreased with the increase in pH values from 1. However, maximum precipitation of protein was increased rapidly from 5 to 85.34% with increasing pH 4 to 1.23. The effect of pH on protein precipitation is presented in Fig. 2. The isolated protein was contained 92.65% protein which is the sign of high purity.

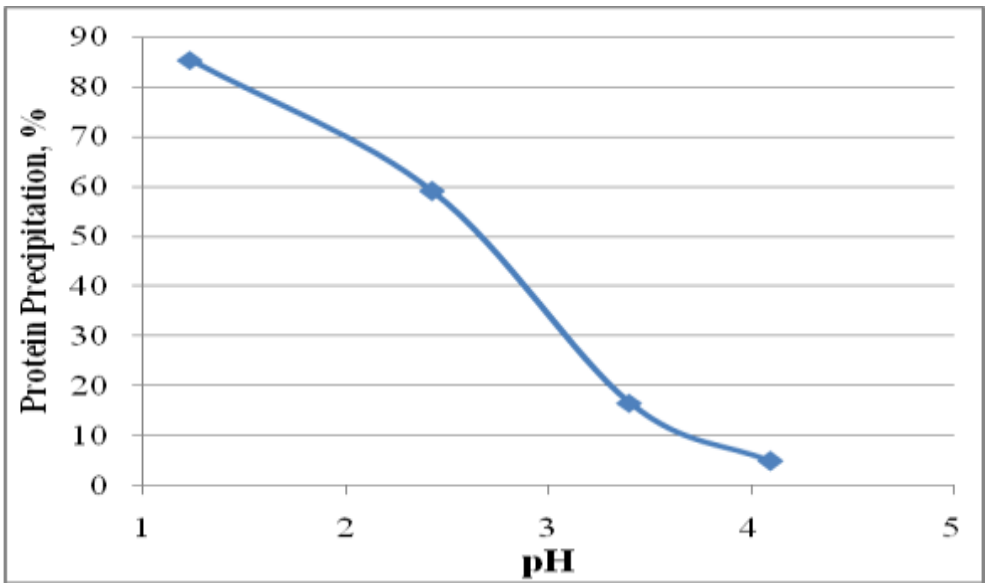

Fig. 2 Protein precipitation at various pH.

Protein Digestibility

The pepsin digestibility of two forms of protein: protein in turmeric, and precipitated protein isolate of turmeric investigated. These two forms of protein were shown significant differences in protein digestibility. In vitro protein digestibility value of turmeric was observed only 68.75% because of the presence of fiber and antinutritional compounds present in turmeric protein. On the other hands, the protein digestibility of isolated protein was increased to 96.13%. The higher protein digestibility of isolated protein assigned the absence of fiber and other antinutritional compounds in isolated protein.

Amino Acids Composition

Amino acid composition of protein isolates is an indicator of their nutritive value. The concentrations of essential amino acids and non essential amino acids of isolated protein were present in the Table 1. The isolated protein contained nine essential amino acids and seven non essential amino acids among of the twenty amino acids. These data showed that the isolated protein was a complete protein. The essential amino acid, Lysine was found maximum and glutamic acid was presented maximum as a nonessential amino acid in the isolated protein.

Table 1: The composition of amino acid in isolated protein.

Essential amino acids		Non essential amino acid	
Amino acid	**Content, %**	**Amino acid**	**Content, %**
Ariginine	2.48	Alanine	2.55
Histidine	1.80	Aspartic acid	5.05
Isoleucine	7.58	Glutamic acid	8.75
Leucine	2.53	Glycine	3.42
Lysine	12.73	Serine	2.29
Methionine	3.28	Tyrosine	3.68
Threonine	2.87		
Valine	1.53		

CONCLUSION

Turmeric has a long history of use, not just as a spice, but also as a healing agent and as a magical herb. It may be concluded that turmeric of Bangladeshi origin contains a great deal of protein and the isolated protein was very high purity. The isolated protein was almost digestible and it can be used as good source of protein as well as a therapeutic agent. The presence of 18 amino acids among of 20 amino acids ensured the complete protein for human uptake. Hence, isolated protein can be considered a ideal source of complete protein and a great potential therapeutic agent.

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