Short communication

In vitro Protein Digestibility of Khesari (*Lathyrus sativus*), Mashur (*Lens culinaris*), and Chhola (*Cicer arietinum*)

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Abstract

Pulse protein concentrates (PPCs) from Khesari *(Lathyrus sativus)*, Mashur *(Lens culinaris)*, and Chhola *(Cicer arietinum)* were prepared by acid precipitation method. The *in vitro* digestibility of these proteins was determined. The mean protein contents in Khesari *(Lathyrus sativus)*, Mashur *(Lens culinaris)*, and Chhola *(Cicer arietinum)* were 29.93%, 23.23% and 20.40% respectively. It was found that the amount of extracted PPC was highest in the case of Mashur (79.25%). However, total protein extracted was the highest in case of Khesari, obviously due to highest protein content of this seed. *In vitro* digestibility test with pepsin and pancreatin showed that there was a significant variability among the PPCs. The most digestible one was Mashur having 95.2% digestibility compared to reference protein casein which was 98.5% digestible. The digestibility of Khesari and Chhola proteins was 93.4% and 89.8% respectively. It was found that heat had profound effect on digestibility.

Key Words : Pulse Protein Concentrate (PPC), *In virto* Digestibility, Pepsin, Pancreatin.

Introduction

Pulses and beans are the major contributors of protein in the diet of people in Bangladesh. So both quality and quantity of the pulse and bean proteins are important. Khesari *(Lathyrus sativus)* Mashur *(Lens culinaris)* and Chhola *(Cicer arietinum)* are the three common and major pulses mostly consumed by the Bangladeshi population. These three major pulses cover 70% of the total pulse production of Bangladesh.

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The major constituents of pulses are carbohydrates, protein, lipid, minerals, and vitamins. The protein contents in pulses varies from one variety to another. The percentage of protein (average) in cereal grain is 5-15% in dry form, whereas in legume seeds it is 20-30% (average)[1]. Seeds, particularly, legume seeds are therefore a rich source of protein for human being. These proteins are rich source of most of the essential amino acids particularly lysine, but usually poor in sulfur containing amino acids methionine and cysteine.

Amino acid composition generally indicates the nutritive value of protein[2]. But the observed protein value by animal assay is often lower than the predicted value for amino acid composition. So nutritive value of protein depends on the complete availability of all the amino acids and requires complete digestion. *In vitro* methods of protein evaluation are useful in screening new protein foods and impact of processing of food[2,3].

An *in vitro* digestion procedure is a convenient and rapid way to assess the bioavailability of protein by enzymatic digestion under model conditions[4].

In this study an attempt was made to prepare protein concentrate from three major pulses produced in Bangladesh. The objective of the present study was to assess the *in vitro* protein digestibility of pulse protein concentrates (PPCs) as an indicator of amino acid availability from these proteins.

Materials and Methods

Khesari *(Lathyrus sativus)*, Mashur *(Lens culinaris)*, and Chhola *(Cicer arietinum)* were obtained from local market. All reagents were of analytical grade.

Protein was extracted from various pulse samples by acid precipitation method[5].

Protein content was obtained by estimating the nitrogen content of the pulse samples and multiplying the nitrogen value by 6.25. This was referred to as crude protein content since the non-protein nitrogen (NPN) present in pulses are not taken into consideration.

Heat treatment of the PPCs were heat treated at 100°C in boiling water bath for 5 minutes. After that, the samples were equilibrated to room temperature for further experiments.

The *in-vitro* digestibility of PPCs was carried out by slightly modified method of Walter and Mark[3]. The amount of undigested protein was measured by Kjeldhal method. The digestibility was determined by using the following formula :

$$\text{Digestibility (\%)} = \frac{\text{Total corrected protein (in 200 mg PPCs) - Undigested protein}}{\text{Total corrected protein (in 200 mg PPCs)}} \times 100$$

Three replications were performed in a completely rendomized design. General linear model (GLM) was used to statistical analysis of data. Least significant difference (LSD) values were used to differentiate mean values[7]. Significance was defined at $P < 0.0.5$.

Results and Discussion

Protein Analysis

There was a significant difference between the total protein content of pulses (Table 1). The total protein content as determined by Kjeldhal method showed that Khesari had highest amount of protein (29.93%) compared to other pulses. According to Roy *et al.*[8] Khesari contains highest amount of protein (about 30%) and provides 354 kcal per 100g. The percentage of total extraction was highest in Mashur (79.25%), followed by 75.8% for Khesari and 67.9% for Chhola. This significant variability in the percentage of extraction can be correlated to the variability in solubility and functional properties of the PPCs. Both Khesari and Mashur had a significantly higher purity level compared to Chhola.

Table 1. Total protein, extracted PPCs, amount of protein, purity of PPCs, and percentage of extraction

Samples	Total Protein (%)	Extracted PPCs (%)	Purity of PPCs (%)	Extraction (%)
Khesari	29.93[a]	25.23[a]	90.01[a]	75.87[b]
Mashur	23.23[b]	20.30[b]	90.68[a]	79.25[a]
Chhola	20.40[c]	16.06[c]	86.30[b]	67.94[c]

[abc]Means followed by the same letter are not significantly diffenent of $P > 0.05$.

In Vitro digestibility

There was a significant variability *in vitro* digestibility of the PPCs. PPC from Mashur was the most digestible one (95.2%). The least digestible was Chhola with a value of 89.5%. The reference protein casein had a digestibility value of 98.0% in the same run (Table 2). There was a significant difference also in digestibility among the PPCs before and after heat treatment. All PPCs showed a significantly higher digestibility after heat treatment (Table 2). This finding indicates that heat has no adverse effect on the digestibility of pulse protein in model condition. In a similar study Haque *et al.*[4] reported that infant formulations containing legume based protein showed marked rise in digestibility after heat treatment. Most of the pulse protein is salt soluble globulin and the major fraction is legumin and vicilin. The difference in digestibility of PPCs may be due to the differences in solubility of PPCs in enzyme-buffer system.

Table 2. In vitro digestibility of PPCs after and before heat treatment

PPCs	Digestibility Before Heating (%) Mean ± SD	Digestibility After Heating (%) Mean ± SD
Khesari (Grasspea)	$93.4^{c} \pm 0.56$	$97.6^{a} \pm 0.62$
Mashur (Lentil)	$95.2^{b} \pm 0.40$	$97.9^{a} \pm 0.60$
Chhola (Cowpea)	$89.0^{d} \pm 1.28$	$96.9^{a} \pm 0.76$
Casein	$98.5^{a} \pm 0.55$	$98.6^{a} \pm 0.45$

Maen is the average of three separate determinations, SD = Standard deviation, ^{a,b,c}Means followed by same letter are not significantly different at $P < 0.05$.

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