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EFFECT OF BOILING ON THE *IN-VITRO* PROTEIN DIGESTIBILITY OF FOUR DIFFERENT PULSES

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Abstract

In-vitro digestibility of mung (*Vigna radiata*), mash (*Vigna mungo*), masur (*Lens culinaris*) and gram (*Cicer arietinum*) in raw and boiled states was undertaken. Mung had highest crude protein content (26.18%). The digestion with pepsin followed by pancreatin indicated a significant decrease, from 33.41 to 49.68%, in the digestibility of all the boiled pulses. The highest digestibility among the unsoaked-raw and soaked-raw pulses was 75% and 78.88%, respectively for gram. In unsoaked – boiled and soaked-boiled pulses highest digestibility was 32.68 and 38.46%, respectively for masur. Soaking the pulses prior to boiling improved digestibility. The hydration coefficients of all the pulses, except gram was inversely related to boiling time, which in turn showed a positive correlation with digestibility.

Introduction

Pulses form an important part of the diet of people living in tropical and sub-tropical regions. These are an excellent source of protein and nutrients, provide significant amount of calories (Anon, 1969) and qualitatively are more important being a rich source of certain important amino acids (Parpia, 1973). The pulses also contain certain toxic substances such as trypsin inhibitors which are responsible for relatively low digestibility in the raw state (Liener, 1973).

Tan *et al.*, (1984) reported that autoclaving for 5 min and quick cooking were most effective methods for improving the *in-vitro* protein digestibility whereas dry heat at 200°C for 30 min and direct boiling without pre-soaking were least effective. There is an optimal level of heating legumes which gives maximum nutritive value of proteins beyond which there is a loss of nutritive value (Evans & McGinnis, 1946). The present communication describes the effect of boiling on the *in-vitro* protein digestibility of raw and boiled pulses.

Materials and Methods

Mung [*Vigna radiata* (L.) Wilczek], mash [*Vigna mungo* (L.) Hepper], masur [*Lens culinaris* Medic] and gram [*Cicer arietinum* L.] obtained from the local market and after removing extraneous materials were: i) pulversied in the raw state; ii) soaked in tap water

for 1 h; iii) boiled to render them palatable enough for consumption; iv) soaked for 1 h prior to boiling. Excepting (i) the pulse samples were dried at 50°C in an oven to a constant weight, ground to a powdery state and stored in a refrigerator.

The samples were analysed for crude protein, true protein and non-proteinaceous nitrogen by Micro-Kjeldahl's method (Vogel, 1962). *In-vitro* digestibility with pepsin and pepsin-pancreatin enzymes was carried out by the method of Akeson & Stahmann (1964). The specific activity of pepsin (BDH Chemicals Ltd. Poole, London) was 1 Anson unit/g and that of pancreatin (E. Merck Darmstadt, West Germany) was 350 FIP units/g. Using the formula of Saunders *et al.*, (1973)

$$\% \text{ protein digestibility} = \frac{\text{N in protein concentrate} - \text{N in undigested fragment}}{\text{N in protein concentrate}} \times 100$$

was calculated.

Digestibility with pepsin: Digests were prepared by incubating 200 mg of powdered pulse sample with 3 mg of pepsin in 30 ml of 0.1 N HCl at 37°C shaking for 3 h. The pepsin solution was activated at 37°C for 10 min before adding it to the sample. At the end of the incubation period, samples were centrifuged at 500 g for 30 min and the precipitates after washing with little quantity of water were analysed for the undigested protein.

Digestibility with pancreatin: Pepsin treated sample was followed by neutralization with 15 ml of 0.2 N NaOH and addition of 8 mg of pancreatin in 15 ml of pH 8 phosphate buffer which was previously activated at 37°C for 10 min. The digests were incubated for 24 h and shaken from time to time. Enzyme blanks were prepared by incubating under the described conditions without protein samples. The samples were subjected to the same treatment as described for pepsin digests.

Hydration Coefficient: Samples 100 g each were soaked separately in 3:10 pulse water for 1 h, blotted dry and re-weighed. Using the formula as described by Hulse *et al.*, (1977)

$$\% \text{ Hydration Coefficient} = \frac{\text{Initial Wt.} + \text{Wt. of water imbibed}}{\text{Initial Wt.}} \times 100$$

was calculated. Ninhydrin test and Micro-Kjeldahl estimations detected the presence of nitrogen content in soaking water. All the experiments were carried out twice.

Results and Discussion

The crude protein of unsoaked-raw mung was highest as compared to mash, masur and gram (Table 1). These results are similar to Ahmed *et al.,* (1975). The crude protein values of soaked-raw or boiled and, unsoaked-boiled were in the same range. The NPN values were determined in order to calculate the amount of true protein by substracting the former from crude protein.

Gram had the highest digestibility among the raw and masur among the boiled samples. A comparison of the digestibility of raw and boiled pulses showed a statistically significant decrease in the digestibility of both the unsoaked-boiled ($t = 25.41; > 1.943$) and soaked-boiled ($t = 17.99; > 1.943$) pules (Table 2). These results are in conformity with of Brochers & Ackerson (1950), who found low digestibility after autoclaving in 9 out of 17 species of legumes investigated. Faki *et al.,* (1984) also reported that boiling did not improve the *in-vitro* protein digestibility of chickpea.

Our digestibility results are contrary to the findings of Ahmed *et al.,* (1975), who conducted *in-vitro* experiments with raw and cooked pulses and evaluated digestibility on the basis of net protein utilization. A larger number of enzymes in the *in-vivo* studies could account for increased values of digestibility as compared to *in-vitro* studies. The decrease in the digestibility of boiled pulses could be attributed to polymerization or coagulation of low molecular weight amino acids and/or proteins caused by heat treatment. The coagulated protein could in turn decrease the digestibility. Tannenbaum (1974) suggested that protein digestibility could decrease via non-enzymatic browning reaction (Maillard Reaction) and thermal cross-linking, resulting in the formation of polymeric enzymes, thus lowering the biological value of a protein.

Aw & Swanson (1985) reported that as a result of extended cooking time the water-soluble tannins of seed coats apparently react with bean protein during heating thus lowering the protein digestibility. In the present investigation, the digestibility of all the

Table 1. Crude protein, true protein and non-proteinaceous nitrogen (NPN) of raw and boiled pulses.

Pulse	Crude protein %				NPN %				True protein %			
	Unsoaked		Soaked		Unsoaked		Soaked		Unsoaked		Soaked	
	Raw	Boiled	Raw	Boiled	Raw	Boiled	Raw	Boiled	Raw	Boiled	Raw	Boiled
Mung	26.0	26.18	26.01	25.99	0.92	0.75	0.90	0.80	22.50	22.80	22.07	22.50
Mash	24.65	24.65	24.54	24.40	0.80	0.80	0.86	0.80	19.0	18.75	19.13	19.0
Masur	25.03	25.23	25.51	25.46	1.06	1.0	0.90	0.92	20.0	19.13	20.40	19.50
Gram	23.20	23.13	23.23	23.20	0.64	0.86	0.66	0.92	20.20	19.50	21.25	18.75

Table 2. Digestibility of the pulses.

Pulse	Digestibility with pepsin (%)				Digestibility with pepsin-pancreatin (%)			
	Unsoaked		Boiled		Unsoaked		Boiled	
	Raw	Boiled	Raw	Boiled	Raw	Boiled	Raw	Boiled
Mung	65.0	27.78	66.0	31.66	66.66	30.0	68.83	32.25
Mash	63.81	18.33	64.10	21.05	65.46	21.67	65.70	22.23
Masur	65.93	28.10	69.41	32.69	67.81	32.68	71.87	38.46
Gram	70.93	23.07	74.71	26.66	75.0	25.32	78.88	33.33

samples with pepsin-pancreatin as compared to that with pepsin alone, increased in the range of 1-7% (Table 2). This could be due to greater number of proteolytic enzymes in pepsin-pancreatin, as pancreatin itself is composed of trypsin, chymotrypsin and carboxy-peptidase.

The results obtained for soaked samples showed that soaking had a beneficial effect on the digestibility of the pulses in both raw and boiled states, which was statistically insignificant ($t = 1.45; < 1.943$) in the former and significant ($t = 1.994; > 1.943$) in the latter case. Molina *et al.*, (1975) reported that the subsceptibility of bean protein to heat damage decreased by soaking treatment prior to cooking. The negative effect of soaking on the protein susceptibility to heat damage could probably be attributed to hydration of protein itself during soaking operation.

The hydration coefficients except that of gram were inversely related to boiling durations (Table 3). These results are comparable to Jackson & Marston (1981) who reported that the differences in the cooking time of fresh and aged samples persisted

Table 3. Hydration coefficient and boiling time of unsoaked and soaked pulses.

Pulse	Hydration coefficient (%)	Boiling times (Min)	
		Unsoaked	Soaked
Mung	108.23	60	39
Mash	102.0	85	70
Masur	119.88	52	35
Gram	136.86	125	90

regardless of bean moisture, and our exception of gram could possibly be attributed to this. The hydration coefficients were further found to be directly proportional to the digestibility for all the pulses, however, in the case of boiled samples this relationship was also true except for gram.

A comparison of the boiling duration and digestibility values revealed that except for gram, the pulses boiled for longer durations had comparatively low digestibility. Moreover the effect of soaking on the boiling duration was observed by comparing the boiling time of the soaked and unsoaked pulses, the latter having a much longer boiling duration than the fomer, indicating a positive effect of soaking. However, the results proved to be statistically insignificant ($t = 1.048; < 1.943$). The negative results on the ninhydrin test carried out on the soaking water indicated that no nitrogen was lost during soaking and hence did not affect the digestibility results of the soaked samples.

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