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EFFECT OF COOKING ON THE ESSENTIAL AMINO ACID CONTENT AND NET PROTEIN UTILIZATION (NPU) OF COMMON PULSES

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Abstract. Five commonly grown pulses, gram (Bengal gram), masur (lentil), mung (green gram), mash (black gram), and arhar (red gram) were evaluated for their total amino acid content; available amino acid content and NPU. Pulses contained 23.87–25.31% protein and sufficient quantities of all essential amino acids except methionine and tryptophan. Cooking of pulses caused losses of amino acids to varying degrees but the NPU of cooked pulses was generally higher than uncooked pulses. Gram showed the highest NPU (61.9%).

Legumes and pulses are important source of edible protein consumed by the people of Pakistan. Bengal gram, black gram, green gram, red gram and lentil are the pulses which are most commonly cultivated in Pakistan over 34 lakh acres and the annual production is about one million tons.[1] The importance of these pulses in the dietaries of the subcontinent has been emphasized by various workers.[2–8] The present investigation was undertaken to find the effect of cooking on the nutritive value of various pulses in terms of microbioassay of nine essential amino acids and by rat growth studies; net protein utilization (NPU).

Experimental

Five dehulled pulses, i.e. Bengal gram (*Cicer arietinum*), black gram (*Phaseolus radiatus*), green gram (*Phaseolus mungo*), red gram (*Cajanus indicus*) and lentil (*Lens esculenta*) were purchased from local market and were divided into two portions. One portion of each of these pulses was cooked according to the conventional methods. The pulses (100 g) were soaked after washing in water for ½ hr before cooking. An appropriate amount of water (500 ml), salt (10 g) and spices (5 g) were added to the soaked pulses and cooked at 100°C till they were soft (20 – 30 min). Chopped onion (15 g) and garlic (5 g) were roasted to light brown in hydrogenated vegetable oil and added to the cooked stuff. The second portion of each of these pulses was not cooked and was treated as control. All the ingredients, i.e. salt, chillies, onions, hydrogenated vegetable oil and water, were mixed in the same proportions as for the cooked portion.

All the samples including control were dried (1 cm thick in 60×40 cm trays) in a cabinet type drier at 50°C for 4–5 hr (to an approximate final moisture content of 8–9%). The dehydrated samples were packed in tin cans and stored in a refrigerator at 6°C.

Proximate Chemical Analysis. Proximate analysis of raw, uncooked (having all the ingredients as the cooked pulses) and cooked samples of pulses were conducted to determine moisture, ash, crude fibre, crude fat and crude protein following the methods of A.O.A.C.[9]

Preparation of Samples

Total Amino Acids Estimation. For the estimation of total amino acid content of pulses except tryptophan, 1.5 g of dried and defatted samples of each pulse was separately hydrolysed in 2N HCl by autoclaving at 115°C for 5 hr. For tryptophan, the samples were digested with 6 N NaOH instead of HCl. The hydrolysate were then neutralized and diluted to 100 ml with distilled water. Finally 10 ml of this stock solution was further diluted to 100 ml with distilled water.

Available Amino Acids Estimation. Twenty ml citrate buffer (pH 7.0) was added to 1.5 g of each of the dried and defatted samples in glass-stoppered test tubes. These tubes were placed in water bath at 56°C and 1 ml of 2% (w/v) solution of papain (in citrate buffer) was added to each sample. After an incubation period of 2½ hr the hydrolysates were diluted to 100 ml with distilled water, 10 ml of this stock solution was further diluted to 100 ml with distilled water.

Microbioassay of Amino Acids

Total and available leucine, isoleucine, histidine, methionine, arginine, valine and tryptophan were determined using the test organism *Streptococcus zymogenes* NCDO 592.[10]

Total and available lysine and phenyl alanine were estimated by using the *Leuconostoc mesenteroides* P60 as test organism. The turbidity was measured using Unicam spectrophotometer SP 600 at 580 μ.

Net Protein Utilization

Protein utilization of the uncooked and cooked pulses was determined using albino weanling rats.[12] Weight gains of albino rats and amounts of food consumed were recorded daily. NPU was calculated

using the formula:

$$\text{NPU} = \frac{B-(BK-IK)}{I} \times 100$$

Where B, mg body N of rats on test diet; BK, mg body N of rats on the non-proteinous diet (control); I, mg N intake of rats on test diet; and IK, mg N intake of rats on control diet.

Results and Discussion

Proximate Chemical Composition. Proximate chemical composition (Table 1) of raw pulses varies within a limited range with the exception of Bengal gram which showed higher values for crude fat and crude fibre. The uncooked and cooked pulses showed an increase in fat content due to the addition of hydrogenated vegetable oil. The values for raw samples are in agreement with the data given in the literature.[6–8]

Total Amino Acids Content. The results for total leucine, arginine, histidine, tryptophan, phenylalanine and lysine content of the uncooked pulses (Table 2) were observed to be in the same range as those reported by several workers.[5–8] However, isoleucine and valine were observed to be slightly lower and methionine content slightly higher than the values reported by those authors except the valine content of Bengal gram which was similar to the reported data.[7] Methionine content of lentil, black gram and Bengal gram were similar to those reported by many workers.[3,4,6,13] Black gram was found to contain very low amount of histidine as compared to other pulses.

Slight variations in total amino acids of these pulses might have occured due to natural and ecological conditions as has been reported.[14]

Effect of Cooking on the Availability of Amino Acids. All the pulses showed variations in the availability of amino acids due to cooking. Available methionine, leucine, isoleucine, valine, lysine decreased in all the pulses. The degree of decrease varied with various pulses as well as particular amino acids. The loss of methionine was maximum in all the samples with the exception of black gram. A decrease of 10–40% of all the amino acids except phenylalanine and lysine was observed in case of black gram. Bengal gram showed losses of avilable leucine, isoleucine, valine, methionine and lysine-content (5–25%) whereas it was appreciably improved in its arginine, tryptophan and phenylalanine content. Lentil showed losses of histidine, tryptophan and methionine-content (8.7–40%) but the available arginine content increased significantly due to heat treatment. Green gram showed the losses of methionine (33%), valine (17%), tryptophan (12.5%), leucine (9%), and isoleucine (4%) while available arginine, phenylalanine and histidine increased by 40, 29 and 4% respectively. Red gram showed maximum losses of histidine, isoleucine and methionine–content ranging from 8.5 to 50% while available lysine, leucine, arginine and tryptophan decreased by 3.3–11.5%. Available phenylalanine content of red gram was found to be increased by 11.5%, as a result of heat treatment.

Available methionine and tryptophan-content of all the pulses were found to be quite low. This was in agreement with the findings of several workers.[3–5,8,13]

Available valine, arginine and histidine-content of all the uncooked and cooked pulses were also observed to be quite low as compared with the total amino acids of these pulses (Table 2). This may be due to incomplete hydrolysis of protein by using enzyme papain, or it may also be due to the presence of spices and hydrogenated vegetable oil which were added to the pulses. Fat might have hindred the activity of enzyme, while salt and spices retard the growth of microorganisms.[9]

The results show that the availability of certain amino acids decreased while of others increased during the cooking of pulses. These changes depended upon the intensity and duration of heat treatment. Proper heat treatment was observed to improve the nutritive value of legumes including soyabean, cotton seed meal, various pulses and beans.[15–17]

Heat denatures proteins. Lipids form oxypolymerisation products. Proteins copolymerise with oxypolymers and also get occluded in them. This decreases availability of amino acids. At the same time heat treatment forms shorter peptides which can easily be attacked by the enzyme. The overall availability of a particular amino acid is a compromise between these opposing factors.

Net Protein Utilization (NPU). Cooking of the pulses was found to have a remarkable effect on the utilization of protein. All the pulses when cooked showed higher values of NPU than those of uncooked pulses (Table 3). Cooked and uncooked Bengal gram were observed to have the highest NPU 61.9 and 57.9% respectively, which is in agreement with the work of Jamal *et al.*[18]

The better NPU of cooked pulses than those of uncooked pulses may be attributed to the fact that proper heat treatment was found to destroy certain toxic factors, e.g. hemagglutinin and trypsin inhibitors, which are commonly present in raw legume seeds and raw pulses. These toxic factors are responsible for toxicity and growth inhibition caused in rats by legume seeds.[19–22] Growth of rat was also inhibited when raw legumes contributed the sole source of protein unless it had been heated sufficiently.[23]

Conclusion

(i) Pulses were found to be rich in protein content ranging from 24.38 (Bengal gram) to 26.31% (green gram).

(ii) Microbiological estimations showed that pulses contain all the essential amino acids in sufficient quantities, specially lysine which is a limiting factor in most of the cereal proteins.

(iii) Tryptophan and methionine content were observed to be slightly deficient in various pulses when compared to FAO reference pattern.

TABLE 1. PROXIMATE CHEMICAL COMPOSITION OF VARIOUS RAW, UNCOOKED AND COOKED PULSES (dry matter basis).

Pulses	Moisture (%)			Crude fat (%)			Crude protein (N×6·25)%			Crude fibre (%)			Ash (%)		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Mash (black gram)	6·50	8·19	8·79	2·16	17·02	16·94	25·63	24·74	24·69	3·65	3·81	3·72	3·41	3·48	3·44
Gram (Bengal gram)	7·24	8·85	9·61	4·59	18·96	18·91	24·38	24·06	23·88	5·67	5·78	5·72	2·82	3·05	2·95
Masur (lentil)	6·98	8·01	7·82	1·92	15·13	14·70	25·81	25·31	24·88	3·15	3·29	3·19	2·75	2·92	2·87
Mung (green gram)	6·87	8·04	10·01	2·11	17·35	17·18	26·31	23·87	23·75	3·28	3·35	3·31	3·01	3·20	3·13
Arhar (red gram)	7·10	8·14	12·60	1·02	16·82	16·68	25·00	24·13	23·81	3·07	3·13	3·21	2·89	3·15	3·09

A, raw; B, uncooked (containing all the ingredients as in cooked pulses); and C, cooked.

TABLE 2. TOTAL AND AVAILABLE ESSENTIAL AMINO ACIDS CONTENT (g/100 g) OF VARIOUS PULSES (average of three readings).

Amino acid	Mash (black gram)			Gram (Bengal gram)			Masur (lentil)			Mung (green gram)			Arhar (red gram)		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Leucine	2·60	1·60	1·24	2·13	1·66	1·46	2·53	1·60	1·55	2·23	1·54	1·40	2·06	1·20	1·14
Isoleucine	1·10	1·02	0·80	1·06	0·95	0·82	1·00	0·90	0·93	0·99	0·97	0·93	1·17	0·58	0·30
Valine	0·66	0·40	0·24	0·93	0·28	0·26	0·66	0·26	0·25	0·70	0·24	0·20	0·80	0·20	0·20
Methionine	0·50	0·17	0·13	0·46	0·08	0·06	0·33	0·05	0·03	0·43	0·03	0·02	0·41	0·02	0·01
Arginine	1·00	0·53	0·42	1·50	0·75	0·90	1·23	0·40	0·66	1·20	0·43	0·60	1·16	0·42	0·40
Histidine	0·30	0·26	0·21	0·63	0·19	0·26	0·60	0·23	0·21	0·76	0·24	0·25	0·83	0·28	0·20
Tryptophan	0·08	0·08	0·05	0·12	0·06	0·08	0·12	0·06	0·05	0·11	0·08	0·07	0·10	0·06	0·06
Phenylalanine	1·20	1·06	1·00	1·50	1·03	1·33	1·46	1·10	1·20	1·80	0·93	1·20	1·86	1·40	1·56
Lysine	1·86	1·43	1·40	1·66	1·48	1·40	1·73	1·46	1·43	1·86	1·53	1·50	1·70	1·50	1·45

A, total amino acids in uncooked; B, available amino acids in uncooked; and C, available amino acids in cooked.

TABLE 3. PROTEIN LEVEL AND NPU OF VARIOUS PULSES.

Pulses		Per cent protein content of diet (dry matter basis)	NPU % (operative)
Mash (black gram)	Uncooked	18·10	43·85
	Cooked	17·43	50·30
Gram (Bengal gram)	Uncooked	17·50	57·90
	Cooked	16·93	61·90
Masur (lentil)	Uncooked	18·75	40·70
	Cooked	16·25	43·10
Mung (green gram)	Uncooked	17·62	42·70
	Cooked	17·56	55·20
Arhar (red gram)	Uncooked	16·50	38·30
	Cooked	17·50	57·30

(iv) Effect of cooking showed losses in the availability of leucine (3–22%), isoleucine (4–48%), methionine (23–50%), valine (4–40%) and tryptophan (12–37%) content of various pulses, due to heat treatment. Available lysine showed very slight decrease (2–8%) during cooking of all the pulses.

(v) Protein utilization of all the cooked pulses were observed to be higher than those of uncooked samples.

(vi) Bengal gram found to have improved in its nutritive value due to heat treatment as shown by its amino acid-content as well as by NPU.

(vii) Generally, pulses were found to have improved in their nutritive values due to the destruction of toxic factors during heat treatment.

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