

Evaluation of Protein Content among Sprouted and Un-Sprouted Seeds of Selected Pulses

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Received date: April 6th 2013; Accepted date: June 20th 2013; Published date: July 1st 2013

Abstract:

The current era of speed and drastically advanced lifestyle Food intake has become one of the major work being neglected and least preferred. This situation resulted in huge cases of Protein deficiency and Malnutrition. In view of the above problems the current work aims to evaluate the protein content among the selected pulses with a focus on comparing the protein content among the sprouted and unsprouted seedlings. The conclusion of the work can propose the pulse which contains maximum protein content that can be readily consumed by substituting it in their diet. The work can be of real use for those individuals who cannot afford to invest much on their diet. The proposed pulses can be substituted to the other non nutritive foods that are much expensive. Based upon the above result the governments free food scheme for school students can be made more nutritive with minimum expenditure. Once the diet of the individual's is redesigned, this can be the first step to solve the nutritive problems faced today.

Chickpea, Hyacinth bean, Black gram, Pea and Mung bean were selected for the study. Protein extraction was performed for both sprouted and unsprouted pulses of all the samples using basic laboratory protocols. The crude proteins were subjected for purification using Salt precipitation followed by Dialysis. The protein content among the samples was quantified using the Lowry's colorimetric method for protein estimation.

Keywords:

Malnutrition, Protein Evaluation, Salt Precipitation, Dialysis, Lowry's method

Introduction:

Pulses are the most traditional form of protein intake in the regular diet of the individuals. The pulses selected in the study are the most commonly used food products all round India. Each pulse is known for its own importance. Pulses, including peas, have long been important components of the human diet due to their content of starch, protein and other nutrients. More recently, the health benefits other than nutrition associated with pulse consumption have attracted much interest ^[1].

Chickpea (*Cicer arietinum* L.) is an important pulse crop grown and consumed all over the world, especially in the Afro-Asian countries. It is a good source of carbohydrates and protein, and protein quality is considered to be better than other pulses. ^[2]Chickpea has significant amounts of all the essential amino acids except sulphur-containing amino acids, which can be complemented by adding cereals to the daily diet.

Mung beans are one of those foods that seem to be loved or hated but this humble food is actually a nutritional powerhouse and may actually be able to be defined as a super food ^[3]. Pulses (dry beans, peas, lentils) are nutrient-dense foods that are recommended as good choices in either the vegetable or meat and alternative food groups ^[4]. Pulses have significant nutritional and health advantages for consumers. They are the most important dietary predictor of survival in older people of different ethnicities^[0] and in the Seven Countries Study, legume consumption was highly correlated with a reduced mortality from coronary heart disease ^[0] Furthermore, pulses are especially high in amylose

starch making them a good source of prebiotic-resistant starch^[10]

Materials and Methods:

Collection of pulses:

Mung Bean, Chickpea, Green Pea, Hyacinth Bean and Black Gram were collected from various mills at Hyderabad.

Fig 1: Showing the Dry Pulses



Sprouting Process:

All the selected sprouts were subjected to sprouting by placing them in moist or damp conditions for approximately 32hrs. The sprouts were initially soaked in water overnight. In 2nd step soaked pulses were bound in wet cotton cloth and placed it for overnight. Sprouting of the pulses was observed in the following day.



Fig 2: Showing the Sprouted Pulses

Protein extraction from the Pulses:

Dry and sprouts pulses were ground into fine powder and paste respectively in the presence of a buffer using motor and pestle. The sample was mixed with the required amount of the buffer and is subjected for centrifugation. The centrifugation is performed at 7000rpm for 10 minutes. The supernatant was collected which constitutes the crude protein.

Purification of Proteins by Salt Precipitation:

The crude extract is collected into a beaker and placed on an ice bath. 44.4% of extract volume of ammonium sulphate was weighed. The salt is added pinch by pinch with the simultaneous and a constant stirring of the crude sample. All the impurities present in the crude extract would be soluble into the ammonium sulphate solution and the pure protein would get precipitated out.

The pure protein precipitate still contains some salt impurities that can be removed by Dialysis. The precipitate is collected into the TE buffer and stored till the next step.

Purification by Dialysis:

The dialysis membrane tubing is taken and tied on one side to be leak proof. The tubing is first activated by heating in distilled water to remove the glycerol bound to the dialysis membrane. The membrane is then heated in distilled water. The step is repeated again and finally the membrane is used to load the sample. The sample dissolved in the buffer is added to the membrane and is sealed by threading. The tubing is checked for leakage. The tubing is then tied to a stand and is suspended in the beaker containing water placed on a magnetic stirrer. The solution and the tubing are allowed to swing for 6 to 8 hours.

After dialysis the membrane is removed from water and then the solution is collected into a fresh tube.

Quantification of proteins:

The pure protein solutions obtained above are subjected for quantification by Lowry's method.

In this method the protein sample is allowed to react with folins reagent and copper sulphate to get a blue colored complex. The color is read in a colorimeter. The concentration of the unknown sample can be obtained from the standard or calibration graph which depicts the absorbance values of known protein samples. Once the concentrations of all the selected pulses are obtained the conclusion of the work can be derived.

Results and Discussion:

Extraction of the crude protein:

All the selected pulses were homogenized using a buffer and collected into different labeled Falcon tubes. The solution is further subjected to centrifugation



Fig 3: The falcon tubes containing the crude extract

The collected extracts were subjected for purification and the final pure proteins were processed for quantification of protein content.

Lowry’s Method for protein estimation:

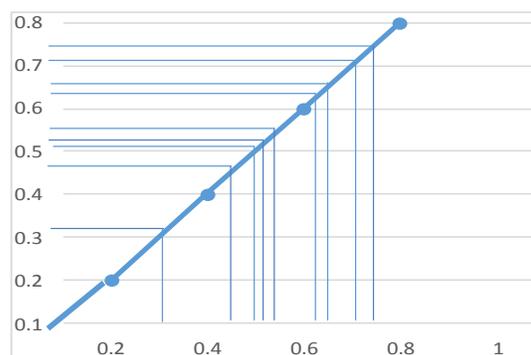
All the protein samples were subjected to protein estimation and the OD values were obtained using the colorimeter. The values were marked against the standard graph. From the graph the concentrations of the unknown samples was calculated as under in the Table 1.

Table 1: Protein concentration in the sprouted and un sprouted pulses of all the selected samples

Sample	Concentration of protein in Un-Sprouted pulse mg/ml	Concentration of protein in Sprouted pulses mg/ml
Chickpea	0.96	0.98
Hyacinth Bean	0.98	1
Black Gram	1	1.03
Pea	0.95	0.98
Mung Bean	1	1

The above table depicts the variability of the protein content among different selected pulses. According to the results Mung Bean and Black gram are known to contain maximum protein content among the selected pulses. Further the values reveal the increase in the protein content upon sprouting for the pulses Pea, Hyacinth Bean and Chickpea. Whereas the Mung bean and black gram did not show considerable variation in the protein content among sprouted and un sprouted pulses.

Fig 4: Graph showing the protein content among the selected pulses:



Conclusion:

From the above work it can be concluded that among the selected five varieties of pulses Black Gram and Mung bean were identified as the sources of high protein content when compared to the other selected pulses. By substituting these pulses to the other non nutritive food in the diet of the individuals the nutritional quality of the food can be improved and this can prevent the Malnutrition and Protein deficiency problems in India. When validated with the clinical studies, the work can be implemented in the diet habits of the individuals.

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