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A REVIEW ON A FEW LEGUMES AND METHODS OF PROTEIN QUANTITATION

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ABSTRACT

Leguminosae (Fabaceae or Papilionaceae) is one of the largest angiospermic families. Consisting of several important plants which are also called as legumes, leguminosae is the third largest plant family. This review presents a report on a few select legumes such as Cicer arietinum (Chick pea), Medicago sativa (Alfa alfa), Vigna mungo (Black gram), and Pisum sativum (Pea). The essentiality of protein contents of the select legumes has been highlighted. There are several methods stated for the protein quantitation such as Biuret, Kjendahl, Bradford, Lowry, Smith, Warburg-Christian, etc. The most recommended and majorly employed protein analysis methods are of Bradford (1976) and Lowry's (1951).

Keywords: Leguminosae, Cicer arietinum, Medicago sativa, Vigna mungo, Pisum sativum, Protein analysis, Bradford, Lowry

INTRODUCTION

Every living being on this planet earth is a product of protein. Legumes are an essential component of human diet as they are rich in protein contents as well as other nutritional elements such as carbohydrates, mineral, vitamins, among others. Legumes are classified under the family Leguminosae.

The angiosperm family of Leguminosae (Fabaceae or Papilionaceae) includes plants which have legume type of fruits known as bean or pea (Ahmad, F., et al., 2016). The family is hence also called the Legume family or the Bean family. Leguminosae is one of the largest families containing thousands of herbs, shrubs and trees (Patel, S., & Shah, D. B., 2014). Leguminosae (Papilionaceae) stands third in rank in regards to the number of genera and species, exceeded by Asteraceae (Compositae) and Orchidaceae (Bairiganjan, G. C., and Patnaik, S. N., 1989). There are around 730 genera and over 19,400 species in this family (Stevens, P. F., 2008).

Fabaceae is most commonly found in tropical rainforests and dry forests in the Americas and Africa (Burnham, R. J., & Johnson, K. R., 2004). It includes trees, shrubs and herbaceous plants perennials or annuals, which are easily recognized by their leguminous fruits and their compound, stipulated leaves (Rahman, A. H. M. M., & Parvin, M. I. A., 2014).

A number of Leguminosae plants and their seeds have been a staple human food for millennia and their use is closely related to human evolution ((Rahman, A. H. M. M., & Parvin, M. I. A., 2014). The seeds of the Fabaceae family have been of particular interest to nutritionists due to its richness in several essential components, especially protein. Given this, they have been recognized as nutritive food (Gulewicz, P., et al., 2014).

Some of the important agricultural plants and food plants classified under the family Leguminosae are Cicer arietinum (Chick pea), Medicago sativa (Alfa alfa), Vigna mungo (Black gram), Pisum sativum (Pea), Arachis hypogaea (Peanut), Glycine max (Soybean), Phaseolus mungo (Bean), Glycyrrhiza glabra (Liquorice), etc. Commonly, these plants are also known as pulses. Although pulses have been consumed for several thousand years (Kerem, Z., 2007), it is only from the past few decades that an elevated interest in pulses as food and their potential impact on human health has revived.

Cicer arietinum

Chick pea (Cicer arietinum L.) an annual plant of the Fabaceae family (Wang, J., et al., 2021). It is an important pulse crop grown and consumed all over the world, especially in the Afro-Asian countries. Its protein quality is considered to be better than other pulses. Currently, chickpea is grown in over fifty countries across the Indian subcontinent, North Africa, the Middle East, southern Europe, the Americas and Australia. Globally, chickpea is the third most important pulse crop in production, next to dry beans and field peas (Jukanti, A., 2012; Boukid, F., 2021).

Chick pea is white or cream in colour, have a rounder shape with a less pronounced beak, and are generally large and comparatively heavier than other pulses (0.2–0.6 g) (Wood, J. A., Knights, E. J., & Choct, M., 2011). In the Indian subcontinent, chick pea is split (cotyledons) as 'daal' and ground to make flour (besan) that is used to prepare different snacks. Chickpeas are of "Kabuli" and "Desi" types (Gupta, R. K., 2017).

The protein content in chick pea significantly varies as a percentage of the total dry seed mass before (17–22 %) and after (25·3–28·9 %) dehulling (Hulse, J. H. (1991); Badshah, A., et al., 2003). Chick pea protein quality is better than some pulse crops such as black gram (Vigna mungo L.), green gram (Vigna radiata L.) and red gram (Cajanus cajan L.) (Kaur, M., Singh, N., & Sodhi, N. S., 2005). Chick peas contain several bioactive compounds that have reported anti-cancerous activity (Gupta, R. K., 2017).

Medicago sativa

Lucerne or Alfalfa (Mielmann, A., 2013) is the most popular and widespread proteinyielding crop which is grown in cool-climate regions (Gaweł, E., 2017). It is a drought, hardy, perennial (Gault et al., 1995), herbaceous forage legume (Lenne and Wood, 2004). Alfa alfa is one of the oldest plants cultivated as forage for livestock feeding for more than 3,300 years (Pioneer Brand Products, 2011). Lucerne (Medicago sativa L.) comprises approximately 100 species. In Poland, farmers call lucerne "the queen of fodder crops" because of its high nutritional value (Gaweł, E., 2017). Some of lucerne compounds are utilized by cosmetics industry. In the human diet, lucerne provides a source of protein, mineral salts, vitamins, macro and micro nutrients and secondary metabolites (Gaweł, E., 2017).

Alfa alfa has a strong and straight ramose stalk that reaches 90 cm in height. The leaves are trifoliate. It has a deep tap root and the flowers are violet to purple in colour. The inflorescence is cephaloid and the fruit is a polyspermous pod with small oval seeds (Gaweł, E., 2017).

Alfa alfa is a great source of carbohydrates, minerals, vitamins, and especially proteins (Tharanathan and Mahadevamma, 2003; Hao et al., 2008; NLO, 2010). Lucerne protein concentrate is a natural antioxidant. It contains 15–22 % crude protein on a dry matter basis (Scholtz, 2008). Lucerne protein is a good source for producing nutritious and functional food (NLO, 2010). The Alfa alfa protein concentrate (APC) is stated as a potential source for high quality of protein for human consumption (D'Alvise et al., 2000). Through fractionation of lucerne, it was demonstrated that alfa alfa contains RuBisCO (Petin and Luzerne, 2010). In addition to essential elements such as proteins, alfa alfa contains high therapeutic value with entopharmacological, phytochemical and therapeutic importance in human nutrition, which is mainly due to the presence of secondary metabolites (Bora and Sharma, 2011).

Vigna mungo

Vigna mungo (L.) Hepper or commonly called as Black gram, belongs to the family Leguminosae (Papilionaceae) (Zaheer, M., et al., 2020). It is an important pulse crop in the semi-arid tropics and subtropical areas (Ramu, P. S., Swathi, K., & Rao, S. G., 2018). Black gram is grown throughout India as a pulse crop and stands fourth in production and acreage in Indian agriculture (Yasmin, K., et al., 2019; Chatterjee, A., Pakrashi, S. C. 1992).

It is an erect hairy annual plant with long twining branches. The flowers are small and yellow in colour and the fruits are cylindrical. The pods are hairy with 1 to 4 seeds in each pod (Zaheer, M., et al., 2020). Some important states in India growing black gram are Andhra Pradesh, Assam, Bihar, Gujarat, Haryana, Maharashtra, Karnataka, Kerala, Tamil Nadu, Madhya Pradesh, Rajasthan, Uttar Pradesh, West Bengal, and Tripura (Singh, K. M. & Singh, R. N., 1977). Black gram is grown on soils inclined to be clayey and on black cotton soil (Khan, F., et al., 2021).

Black gram is very important part of Indian food (Dhumal, J. S., et al., 2019). It is mainly used for the preparation of various food products in the form of cotyledon or daal. The Vigna

mungo seeds are characteristically sweet, with laxative, aphrodisiac, tonic, diuretic, galactagogue and styptic properties (Dhumal, J. S., et al., 2019). The protein contents in black gram ranges from 12 to 42%. Black gram is a great source of protein, fibre, vitamins, calcium and iron (Reddy et al., 1982; Salunkhe et al., 1985). On studying the characterisation of black gram seed storage proteins, the dehulled and defatted black gram flour reported to contain 25% protein like globulins (63%), albumins (12%), glutelins (21%), and a trace amount of prolamines (1%) (Mahajan, R., et al., 1988).

Pisum sativum

Pisum sativum (L.) or more commonly called as Peas are grown around the world for human and animal consumption (Dahl, W. J., 2012). Pea was the original model used in Mendel's discovery (1866) of the laws of inheritance, making it the foundation of modern plant genetics (Smýkal, P., et al., 2012). Pea protein is considered to be hypoallergenic, and many studies highlight its health benefits (Ge, J., et al., 2020).

Pisum sativum is an annual climber where stem is hollow that grows up to 2–3 m. It has alternate, pinnately compound leaves and has 2–3 pairs of 1.5–8 cm long large leaf-like stipules. Flowers are pentamerous with green fused sepals and white to reddish-purple petals of different sizes. Fruit is leguminous with 2.5–10 cm long pod. The pod is a two sealed valves that splits along the seam which connects the two valves. Seeds are round, smooth, and green colour.

Peas have multiple good qualities like high nutritional value, availability and relatively low cost in commercial market (Guindon, M. F., et al., 2021). P. sativum can be eaten in raw form as well as cooked or frozen form (Rungruangmaitree, R., & Jiraungkoorskul, W., 2017). Pea is widely used in crop rotation, which is highly beneficial for the soil as it is helpful in establishing subsequent crops without ploughing (Fischer, E., et al., 2020). In some parts of the world, peas are considered as the main source of proteins with protein content ranging from 190 to 300 g kg^{-1} in commercial varieties (Iqbal, A., et al., 2006). Pea seeds contain about 22-23% proteins. The majority of it is globulins and albumins (Tsoukala, A., et al., 2006). Nowadays, pea protein isolates are most promising alternative to soy protein products (Barać, M., et al., 2015).

As a techno-functional ingredient, the pea proteins are used as flour, concentrates, as well as isolates. Pea flour is prepared from the dehulled and milled pea seeds (Barać, M., et al., 2015).

Protein Quantitation by Bradford's Method

Although there are many methods for protein assay available such as Biuret, Kjendahl, Lowry, Smith, Warburg-Christian, etc., the spectrophotometric assay of Bradford (1976) is majorly recommended by authors worldwide because of its multiple advantages as compared to the other methods (Snyder and Desborough, 1978; Berges et al., 1993).

In Bradford's method of protein analysis, the absorbance is measured at 595 nm in a spectrophotometer after an incubation of minimum 5 minutes to maximum 1 hour depending upon the stability of the protein-dye complex. The net absorbances for each albumin standard are then subjected to regression analysis (known concentration vs. absorbance). The protein concentration for each unknown sample is calculated by the resulting equation. Final protein concentrations of the samples are determined according to the different dilutions made during the extraction procedure (Bonjoch, N. P., & Tamayo, P. R., 2001). The concentration is generally expressed in mg/mL.

The Bradford protein reagent is prepared in distilled water with different components such as Coomassie® Brilliant Blue G-250, Ethanol (95%), and Phosphoric acid (85%).

Protein Quantitation by Lowry's Method

The Lowry's Method of protein analysis is based on both Biuret reaction and the Folin-Ciocalteau reaction. The reactions result in a strong blue color, which depends partly on the tyrosine and tryptophan content (Waterborg, J. H., 2009).

The method uses 2N NaOH, 1N Folin reagent and a Complex-forming reagent which is prepared by dissolving 2% Na_2CO_3, 1% $CuSO_4 \cdot 5\ H_2O$ and 2% sodium potassium tartrate in

distilled water. In this method, BSA (Bovine Serum Albumin) is used as the standard protein (Waterborg, J. H., 2009).

In the protein assay by Lowry's method, 0.1 mL of 2N NaOH is added to the BSA standard or the sample extract and kept in a water bath for about 10 minutes. After this hydrolyzate has cooled down, 1 mL of the complex-forming reagent is added to the series. The complex-forming reagent should always be freshly prepared. After 10 minutes, 0.1 mL of Folin reagent is added and the series is left to incubate for minimum 30 mins and maximum 1 hour. The absorbance is measured by a spectrophotometer at a wavelength of 750 nm (Waterborg, J. H., 2009).

CONCLUSION

It can be concluded that protein is a very essential element of the human diet which can be accessed from several types of legumes. The legumes such as chick pea, black gram, and pea are easily available for human consumption which are a great source of protein contents. Alfa alfa is majorly a livestock crop but through its protein assays in the recent years, it is proposed to be included in human diet. Hence, protein quantitation becomes a crucial step in studying plants for better human health. While there are several methods proposed in literature to study the protein contents of plants, Bradford's method of protein analysis and Lowry's method of protein analysis are the most used.

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