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Review Article

An Overview of Importance and Nutritional Evaluation of Food Protein Quality

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Abstract

Protein is the buzz word commonly being used to market a food product or to emphasize the importance of a particular food. However, the quality of each protein is different in terms of its bioavailability or chemical score based on the presence or absence of essential amino acids. Generally, plant-based proteins lack one or more essential amino acids and need to be complemented by consuming variety of other plant proteins while animal proteins are considered as complete protein due to their high bioavailability and presence of all essential amino acids. Different methods are employed to evaluate the quality of food protein viz., digestibility coefficient, biological value, true and apparent protein digestibility, net protein utilization, protein efficiency ratio, amino acid scores, and in-vitro protein digestibility. The present paper highlights these methods as have been commonly employed to assess the importance of food items in terms of its protein quality.

Keywords: Nutritional; Evaluation; Food Protein; Quality

Introduction

Protein is important for the growth and maintenance of body. Protein-rich food constitutes an inevitable part of balanced diet of general population as well as athletes [1]. There are several foods which are considered rich sources of complete protein like meat, milk and eggs. Among the plant sources, mushrooms, pulses and legumes such as soyabean, peas, and beans provide good amounts of protein particularly to the diet of the vegans [2]. Further, with processing like drying, the proteins get concentrated to as high level as 35% in case of mushroom powder [3], 28.8% in partly skimmed milk powder [4] and 19.3% in finger millet and moringa nutritious bar [5]. Nowadays, new products are being launched in the market with novel sources of protein like single cell protein or combination of different protein rich raw materials to meet the growing demands of health-conscious consumers. Their suitability for the protein quality is a question mark unless tested in the laboratory following standard protocols. This is particularly relevant for the emerging plant-based milk and meat alternatives as they involve extensive processing during their manufacture [6]. Food processing has various effect on the properties of protein due to certain changes like proteolysis, protein cross-linking, amino acid racemization and Maillard reaction [7]. Further, the food product may show difference in the bioavailability of protein after the process of digestion in the body. The process of digestion reduces large food molecules (for example, proteins, lipids, nucleic acids, and starches) into its chemical building blocks, which are then absorbed by the lining of the alimentary canal to nourish the cells of the body. It involves hydrolysis of food components by enzymes, bile acids and digestive juices. After absorption of simpler molecules, the digested nutrients get transported to bring biosynthetic reactions, wherein larger molecules are synthesized from smaller constituent, using ATP molecules as the source of energy to build bone, muscle mass, and new proteins, fats, and nucleic acids contributing to growth and repair of body. During digestion, absorption and utilization, proteins behave differently affecting their nutritive quality.

Protein quality evaluation methods

There are various methods to judge the quality of food protein based on their bioavailability or chemical score. Determination of bioavailability of proteins depends upon various parameters such as weight gain, nitrogen absorption and retention, or other measures of physiological performance of animals (preferably rats) or human models fed controlled diets containing almost equal quantities of different proteins. It is also clear that these differences are in most instances related to the amino acid composition of the proteins since additions of essential amino acids to proteins often greatly improve their nutritive value [8]. Earlier, calculations of the protein requirement were done when proteins of differing quality were consumed, and if the requirement for one particular protein that is maximally utilized was known [9]. A correction factor was obtained and the quantity for other diets having proteins of lower quality were then calculated by multiplying with this value. For example, if the protein requirement of maximal quality for an individual of a certain weight, age and gender is X, the requirement would be 2X when the dietary protein is only 50% utilized, 4X when the dietary protein is only 25% utilized, etc. This method assumed that the nutritive quality of proteins varies in a linear fashion from 0 to 100. Various protein quality evaluation methods are given in AOAC (2000) [10] such as section 45.3.04 (AOAC Official Method 960.48, Protein Efficiency Ratio), section 45.3.05 (AOAC Official Method 982.30, protein Efficiency Ratio, Calculation Method) section 45.3.06 (AOAC Official Method 991.29; True Protein Digestibility of Foods and Food Ingredients, Rat Bioassay) and section 45.4.04 (AOAC Official Method 988.15, Tryptophan in Foods and Food and Feed Ingredients). Some important methods are briefed as below.

Digestibility Coefficient

Digestibility coefficient is measured directly by subtracting the amount of protein in the feces from those provided in the feed to

$$DC = \frac{I-(F-Fm)}{I} X\ 100$$

xample, rat.

Where F-Fm is the food nitrogen lost in digestion through feces

I = Nitrogen intake of test protein

It gives an idea about the daily requirement of protein to balance the losses of nitrogen from the body and to maintain the lean body mass.

Biological value and protein digestibility

Biological value has been defined as the percentage of absorbed nitrogen retained in the body. A complete evaluation of the dietary protein quality involves measurement of both the Biological Value and the Protein Digestibility. These values are obtained by measuring the fecal and urinary nitrogen when the test protein is fed and correcting for the amounts excreted when a nitrogen-free diet is

$$BV = \frac{I-(F-F0)-(U-U0)}{I-(F-F0)} X\ 100$$

where

- I = Nitrogen intake of test protein
- F = Fecal nitrogen
- Fo = Fecal nitrogen on nitrogen-free diet (Metabolic N)
- U = Urinary nitrogen
- Uo = Urinary nitrogen on nitrogen-free diet (Endogenous N)

The basic assumption made in the measurement of Biological Value is that the endogenous N and metabolic N are constant values and are subtracted from the test values as shown in the equation. Apparent digestibility and True Protein Digestibility (TPD) can be determined by AOAC 991.29 (2000) methodology [10].

$$\text{Apparent digestibility, \%} = \frac{Ni - Nf}{N_i} \times 100$$

$$\text{True protein digestibility (TPD), \%} = \frac{Ni - (Nf - Nm)}{N_i} \times 100$$

where

- N_i = Nitrogen intake [= Diet consumed (g) x Nitrogen content of the diet (mg/g)]
- N_f = Fecal nitrogen = [Weight of feces (g) x Nitrogen content of feces (mg/g)]
- N_m = Metabolic nitrogen (for the test diet group)

From the rat balance method, high values of 93-100% for true protein digestibility (TPD) were observed for animal foods or food products such as casein, beef, skim milk, tuna fish, chicken products and soy protein isolate [11]. Chick peas, oats, wheat, and pea protein concentrate have TPD values in the range of 86-92% while values below 85% were reported for different types of dry beans and millets. Country wise, food habits of people are different and so the type and content of protein varies in the diet. TPD of diets from developing countries was found to be between 54-78%, which was lesser than that of protein in North American diets (88-94%) [11]. Nutrient binding is mainly linked to the presence of some anti-nutritional factors like oxalates, tannins, Maillard reaction compounds and trypsin inhibitors particularly in the unprocessed food [12-15]. It is of vast concern to the countries where the problem of protein-energy malnutrition (PEM) is prevalent resulting in high mortality of children, affecting their learning abilities and work. Therefore, novel products with increased content of protein with the incorporation of protein-rich ingredients like mushroom powder or whey protein concentrate or whey and dried moringa leaves have been suggested by Kumar., *et al.* (2019), Arora and Patel (2017), Arora (2022) and Jaiswal., *et al*. (2023), respectively [3,5,16,17].

Net protein utilization (NPU)

NPU is defined as the % of the dietary protein retained. Like Biological Value, NPU estimates nitrogen retention but in this case on determining the difference between the body nitrogen content of animals fed no protein and those fed a test protein, the value is divided by the amount of protein consumed.

$$NPU = \frac{\text{Retained nitrogen}}{\text{Intake of nitrogen}} \text{ X } 100$$

Since both NPU and BV are based upon estimates of "retained nitrogen", they should measure the same thing except that in the calculation of NPU the denominator is the total nitrogen consumed whereas in the calculation of BV it is the amount absorbed [1]. BV is generally expected to be higher than NPU because of the amount of nitrogen lost owing to poor digestibility or absorption.

Protein efficiency ratio

Protein Efficiency Ratio (PER) has been the method most widely used because of its simplicity. It accounts for the gain in body weight per gram of the type of protein consumed. It is normally conducted using growing rats as the model. A comparison of the values of B.V., NPU, PER is shown in Table 1. These values are higher for animal source of protein than the plant sources.

$$PER = \frac{\text{Gain in body weight (g)}}{\text{Protein intake (g)}}$$

Food	B.V.	NPU	PER	Limiting Amino Acid*
Egg	100	96	3.8	Nil
Whey Protein	104	92	3.2	-
Milk	90	85	2.8	SAA
Meat	74	76	3.2	SAA
Fish	80	74	3.5	Tryp
Rice	80	77	1.7	Lys, Thr
Wheat	66	61	1.3	Lys, Thr
Maize	50	48	1.0	Lys, Thr, Tryp
Bengal gram	74	61	1.1	SAA
Soy protein	74	61	2.2	
Red gram	72	54	1.7	SAA, Tryp
Groundnut	55	-	1.8	Lys, SAA, Thr
Gingelly seeds	62	-	-	Lys

Table 1: B.V. (Biological value), NPU (Net Protein Utilization), PER (Protein Efficiency Ratio) and Limiting Amino Acids for some food products.

Source: Gopalan, C., B.V. Rama Sastri and S.C. Balasubramaniam, 1996, Nutritive value of Indian Foods, NIN, ICMR, Hyderabad [2] and [21].

*Swaminathan M., 1988. Essentials of Food and Nutrition, Bappco, Banagalore [18].

SAA: Sulphur Amino Acids

Amino acid scores

A chemical grading of the quality of a protein can be made by comparing its amino acid content with that of a reference protein. The FAO/WHO (2001) has suggested amino acid pattern of reference based on needs for each amino acid [19]. Hen's egg protein is taken as the reference protein because it contains all essential amino acids in adequate amounts. Several methods based on chromatography, chemical or microbiological techniques are used for estimating the amino acid content of proteins.

$$\text{Amino acid score} = \frac{\text{mg of amino acid in 1g test protein}}{\text{mg of amino acid in 1g reference protein}}$$

In practice, the scores need to be calculated only for lysine, the sulphur containing amino acids (SAA) viz. methionine and cystine and tryptophan, as one or other of these is the limiting amino acid in common foods. WHO (2007) has recommended to do corrections in amino acid scores for true protein digestibility [20].

PDCAAS (Protein digestibility corrected amino acid score)

The WHO (2007) and the US FDA adopted Protein Digestibility Corrected Amino Acid Score (PDCAAS) as the official assay for evaluating protein quality [20]. The PDCAAS is based on amino acid requirements of children (age 2-5 years). This represents the amino acid score after correcting for digestibility. Proteins that after correcting for digestibility, provide amino acids equal to or in excess of requirements receive a PDCAAS of 1.0 [20]. This system has improved the rating for soy protein to PDCAAS 1.0. However, the corrected amino acid score reduces the importance of high-quality protein like milk protein in compensating the amino acid composition of inferior quality protein like wheat or rice which has low concentration of essential amino acid lysine [9]. Usually, to improve the nutritional and functional quality, high quality milk products such as milk powders or whey protein concentrate is used to develop new products [16]. PDCAAS values for some of the common food products are shown in table 2. For extensive understanding, one can refer the review paper by Boye., *et al.* (2012) [21], which gives an excellent compilation of protein quality comparison of different food products.

$$PDCAAS = \frac{\text{mg of limiting amino acid in 1 g of test protein}}{\text{mg of same amino acid in 1 g of reference protein}}$$

$$\text{X fecal digestibility percentage.}$$

Amino acids that move beyond the terminal ileum in the body are less likely to be absorbed for use in protein synthesis. They may pass out of the body, or may be absorbed by bacteria, thus will not be present in the feces, and will appear to have been digested. The PDCAAS takes no account of the location where the proteins have been digested. Similarly, amino acids that are lost due to anti-nutritional factors present in many foods are assumed to be digested according to the PDCAAS. Milk protein has very high digestibility close to egg protein. The amino acid score for casein is 1.19 and is normally corrected as Protein Digestibility Corrected Amino Acid Score (PDCAAS) of 1.00 [24]. In 2013, the FAO proposed changing to Digestible Indispensable Amino Acid Score (DIASS) [8].

Food	Ref. [22, 2]	Ref. [23]
Soya protein	1.0	82
Milk powder/Milk	1.0	100
Casein and Whey	1.0	-
Egg/ Egg white	1.0	100
Beef protein	0.92	100
Pea protein	0.73	79
Rolled oats	0.57	-
Peanut meal	0.52	-
Lentils	0.52	-
Rice	0.47	-
Whole wheat	0.40	48
Potato	-	93

Table 2: PDCAAS (Protein Digestibility-corrected Amino Acid Score) values for some food.

Source: Srilakshmi, B. 2014. Nutrition Science (4th revised edition) New Age International Publishers, India [22]; Gopalan, C., B.V. Rama Sastri and S.C. Balasubramaniam, 1996, Nutritive value of Indian Foods, NIN, ICMR, Hyderabad [2] and Hambræus, L. 2014. Protein and Amino Acids in Human Nutrition. *Reference Module in Biomedical Sciences*. Elsevier [23].

DIAAS (Digestible indispensable amino acid score) or IAA (Indispensable amino acid score)

Amino acids such as His, Ile, Leu, Lys, Met, Phe, Thr, Trp and Val are indispensable essential amino acids which need to be essentially present in the diet of an individual to remain healthy as they cannot be synthesized in the body. A new protein quality measure digestible indispensable amino acid score (DIAAS) is recommended to replace PDCAAS. The DIAAS is defined as

$$\text{DIAAS\%} = \frac{100 \times [(\text{mg of digestible dietary indispensable amino acid in 1 g of the dietary protein})}{(\text{mg of the same dietary indispensable amino acid in 1g of the reference protein})}$$

As protein digestibility does not truly reflect the value in terms of available individual dietary indispensable amino acids, therefore, DIAAS method is preferable.

There are three distinct uses of the DIAAS, first in calculating quality of protein in mixed diets for meeting the needs of daily requirements for protein of an individual [21]. Second, to realize the importance of individual protein sources with higher values in complementing less nutritious proteins and third, in regulating and controlling protein-based food market for securing the interest of consumers. Recently, consumption of wide variety of plant proteins has been suggested to have high quality protein in the diet based on consumer preference and availability [25].

In-vitro protein digestibility (IVPD)

The free amino acids (FAA) released (%) during digestion with respect to the amount of crude protein undigested can be calculated by in-vitro methodologies using lesser time and efforts. It may be based on pH adjustments and use of gastric enzymes. Generally, such methods try to simulate the food digestion system in lab. In one of the methods, trichloro acetic acid (TCA) is used to extract the soluble protein, it is then hydrolyzed using appropriate digestion fluids and the free amino acids (both essential as well as non-essential) released in the bioaccessible fraction are measured using GC coupled FID (Flame ionization detector) [26-29].

$$\text{Free amino acid (FAA) release (\%)} = \frac{\text{gm FAA in the bioaccessible fraction} \times 100}{\text{gm crude protein in sample}}$$

IVPD for less known foods like Amaranth (*Amaranthum*) has been reported to range between 78.8 to 82.0% while that for Chia (*Salvia hispanica*) between 49.4 to 77.53% [30-32]. There is an increasing trend for plant-based diets with variety of legumes, nuts, leaves, cereals, millets and seeds to realize health benefits as well as to protect the environment [33,34]. There is a growing interest among scientists to determine IVPD in lab, however, for such unconventional food products, protein digestibility should also be measured by in-vivo methods [21].

Conclusion

It is important to determine protein quality of new products developed in food R and D labs with novel sources or combinations of proteins before laying any recommendation to the consumer. Knowledge to determine the quality of protein has evolved to the amino acids level. Some methods involve determination of protein quality based on essential amino acids while other still prefer estimating bioavailability using animal models. The determination of true protein digestibility is based on rat-study and gives the true value of protein bioavailability in terms of absorption and retention of nitrogen generally done for major food groups like staple cereals and milk, however, in-vitro methods are quick and is applied for determining protein quality of new or lesser utilized food items like chia seeds. Nonetheless, the importance of milk protein and egg protein remains invariably high in all the tested methods, therefore are advised to form the regular part of the diet of lacto-ovo vegetarians.

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