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In vitro chemical procedures to estimate amino acid digestibility in soybean

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Abstract

Protein digestibility is a major index of protein quality because a certain amount of amino-acids may be present in a food and it may not necessarily be available to the organism to provide nourishment safety, quality and public health. A simple, fast and inexpensive chemical method for protein digestibility determination is the KOH-solubility test. The protein that is soluble in 0.2% KOH is the indigestible fraction and therefore the digestible quantity of protein may be calculated as the difference between the protein in the sample and the soluble protein. The percentage of soluble protein from total protein decreases to 60% when the proteins are denatured.

Keywords: protein, digestibility, soybean

1. Introduction

Soy bean (*Glycine max)* is an important source of oil (17-25%) and protein (35-45%). It contains large amounts of Vitamin B1 and B2 but it is rather low in vitamin C. The crude protein of soybean meal ranges from 41 to 50% (dry matter basis) depending on the amount of hull that is removed, and the processing method used (Liener, 1994).

The majority of soy proteins are reported to be globulins and these are extractable in water and dilute salt solutions, but are insoluble in water at their isoelectric point, pH = 4.2 - 4.6 (Liu, 1997; Stauffer, 2002; Hou and Chang, 2004). This phenomenon is used during the preparation of protein concentrates, isolates and curd making from soy milk. Soy protein globulins are further divided into two classes, legumin and vacilin, which have been given trivial names, namely glycinin (11S) for the legumin and conglycinin (7S) for the vacilins. These names have been derived from the genus name for soybean plant, *Glycine*.

Solubility is one of the most basic physical properties and prime requirements in functional food systems (Liu, 1997). The hydrodynamic behavior and protein–protein interactions depend basically on the degree of dissociation, denaturation and aggregation of the major storage soy protein globulins, 7S and 11S.

While the amino acid profile pattern is probably the main determinant of protein nutritional quality, the digestibility of a protein and bio-availability of its constituent amino acids are the next important factors (Oshodi et. al., 1995). This is true because proteins are digested, absorbed and utilized to different extents. Protein digestibility is defined as the percentage protein absorbed after ingestion of a certain amount of protein by humans or animals (Oshodi et. al., 1995; Liu, 1997). It is closely related to amino-acid availability.

Protein digestibility is a major index of protein quality because a certain amount of amino-acids may be present in a food and it may not necessarily be available to the organism for nourishment.

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This means proteins cannot be utilized unless they are digested.

The differences in protein digestibility are brought about by the susceptibility of a protein to enzymatic hydrolysis in the digestive system and this is directly related to the primary, secondary and tertiary structure of the protein (Liu, 1997).

The problem of relatively low protein digestibility in legumes deserves attention. Although proteins of plant origin offer a considerable protein source for alleviating the shortages of food protein facing the greater part of the population, Liener (1982) pointed out that many plants contain anti–nutritional factors which affect protein quality, and Bressani et.al. (1982) also implicated legume seeds to contain proteins that inhibit the proteases of the mammalian digestive tract, notably trypsin and chymotrypsin.

2. Material and methods

The protein solubility was determined according to the procedure of Araba and Dale (1990). The procedure involves the incubation of a sample with a 0.2% KOH solution for 20 min at room temperature. Following this incubation, the sample is centrifuged and the supernatant is analyzed for the nitrogen concentration. The nitrogen that is left in the supernatant is the indigestible fraction.

The digestible quantity of protein may be calculated as the difference between the protein in the sample and the protein in the supernatant. The protein was determined by the Kjeldahl method using the Kjeltech system.

The solubility of the protein, expressed as a percentage was calculated by dividing the protein content of the KOH-extracted solution by the protein content of the original soybean sample.

3. Results and discussion

Solubility is one of the most basic physical properties and prime requirements in functional food systems. The hydrodynamic behavior and protein–protein interactions depend basically on the degree of dissociation, denaturation and aggregation of the major storage soy protein globulins, 7S and 11S. For optimum functional properties, a solubility index above 90% is required. Good protein solubility generally correlates with optimum gelation, emulsifying and foaming ability of the protein (Lakemond et al., 2000). Solubility of proteins decreases at ionic concentrations of less than 0.1M and increase above this concentration. This is caused by the decreased electrostatic repulsion and enhanced hydrophobic interaction of protein molecules as a result of electrostatic shielding of charge groups in proteins by ions. Solubility increases sharply below and above their isoelectric point (pH 4.2- 4.6) (Liu, 1997).

Table 1 shows the variation of unprocessed and processed (heated for 20 minutes at different temperatures) soybean protein solubility in KOH.

The main reason for these variations is differences in processing technology and procedures. The differences in protein solubility are directly related to the primary, secondary and tertiary structure of the protein.

Cooked (denatured) proteins are less soluble than raw proteins. Solubility therefore gives an indication of the extent of cooking. The heating temperature is negative correlated with the protein solubility (Figure 1).

Table 1. Effect of heating temperature on the protein solubility

Heating temperature (°C)	Soluble protein %	Soluble protein from total protein %
20	40.46	92.60
40	40.37	92.40
60	36.56	83.66
80	27.27	62.40
100	26.69	61.07
120	26.20	59.95

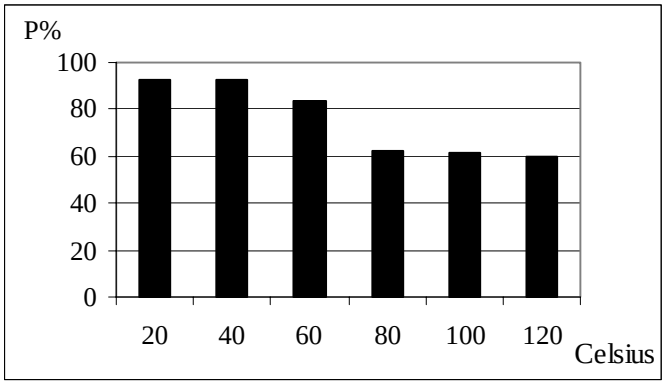

Figure 1. The proportion of the soluble protein from the total protein at different heating temperatures

4. Conclusions

The protein that is soluble in 0.2% KOH is the indigestible fraction and therefore the digestible quantity of protein may be calculated as the difference between the protein in the sample and the soluble protein

Protein solubility in KOH decreases with increasing the temperature due to protein denaturation.

The percentage of soluble protein from total protein decreases to 60% when the proteins are denatured.

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