



ISOLATION, CHARACTERIZATION, ANALYSIS AND STABILITY STUDY OF SOYA PROTEINS IN SIMULATED FLUIDS

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ABSTRACT:

The soyabean is an important dietary component which contains abundant amount of proteins or amino acids required by human body for normal growth and maintenance. In this project, isolation of soya proteins is carried out by extraction with 0.01N sodium bicarbonate and thereafter homogenization, centrifugation and dialysis of extract against Tris buffered saline solution (pH 8.8). These proteins are characterized qualitatively by electrophoresis and quantitatively by Biuret assay method. Biuret assay method effectively quantifies the amount of protein in a given sample. Amino acids present in the isolated protein were identified and quantified using standard or marker amino acids. The stability study of isolated soya proteins is carried out in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), prepared according to protocol specified in USP. These proteins were quantified by Biuret assay method after respective time interval in above specified fluids. The soya proteins are found to hydrolyze in SGF after 5 minutes and in SIF within one hour. Thus *in-vitro* stability study of proteins in simulated fluids is an effective approach and alternative to animal model for bio-stability assessment.

Key words: Stability of soya protein; Simulated Intestinal Fluid; Simulated Gastric Fluid; soya bean

1. INTRODUCTION

Gross chemical composition of food includes cereals and cereal products, legumes and oil seeds, fruits and vegetables, meat, fish and their products, milk and dairy products. Composition of food constituents includes proteins, carbohydrates, lipids, enzymes, water and minerals. Among all the ingredients present in food, proteins are an essential nutrient for humans as they play an important role in the structure and function of living organisms. Proteins are available

from different food sources such as seafood, white-meat poultry, milk, cheese, yogurt, eggs, beans, pork tenderloin, soy, lean beef.^[1] Soya bean is the most commonly consumed crop in the world as it contains approximately 40% protein, 35% carbohydrate, 20% lipid, 5% ash. Soy protein is a 'complete protein' as it provides all essential amino acids for human nutrition like lysine and methionine.^[2] United States Food and Drug Administration approved the claim that 25 g of soy protein in a day, as a part of diet low in saturated fat and

cholesterol, may reduce the risk of heart disease. This was done by different long-term studies on the effect of soy protein on cardiovascular diseases. [3] In addition to that they have also shown antihypertensive activity, antihyperlipidemic activity, prevention against osteoporosis, cancer. [3, 4]

Soya protein needs to go through thermal treatment to inactivate anti-nutritive factors such as trypsin inhibitor. Jookyeong Lee optimized the hydrolysis conditions of soy protein for enhanced solubility, thermal stability and bioactivity, maintaining low degree of hydrolysis (DH); in that they determined the solubility and thermal stability in solution of hydrolysates and soy protein isolate at different protein concentrations. Hydrolysis affects thermal stability of soya protein at pH 4.5 and below pH 3.5; soya protein hydrolysates displaced greater and lower heat stability respectively in comparison to soya protein isolates. [5]

In this project, stability studies of soya proteins in simulated gastric and intestinal fluids were carried out so as to identify their metabolism profile in stomach and intestine and thereby compare it with *in vivo* study data.

2. MATERIALS AND METHODS

Materials - Sodium bicarbonate (NaHCO_3), Sodium chloride (NaCl) were purchased from Merck Life Science private limited. TRIS buffered saline pH 8.8, Pepsin and Pancreatin

were obtained from Himedia, Monobasic Potassium Phosphate was purchased from Loba Chemie Pvt. Ltd.

Instruments - UV-Visible Spectrophotometer (Jasco model V- 530), Electrophoresis (Biotech R and D laboratories) Centrifuge (Remi Manufacturer, Model No- C- 851/8)

2.1. Preparation of TRIS buffer saline solution – Accurately 6.05gm of TRIS and 8.76 gm of sodium chloride was dissolved in 800 ml of distilled water. The pH was adjusted with 1M HCl and the volume was made up to 1 liter with distilled water. This solution remains stable for 3 months at 40°C.

2.2. Extraction process of soyabean

Soyabeans were crushed and extracted using 0.01N NaHCO_3 . The solution was centrifuged at 2000 rpm for 5 min. Lipids were extracted with chloroform and dialysis was carried out against TRIS buffer saline (pH 8.8) using membrane space (10000 kg Dalton), in which the protein was released into buffer solution.

2.3. Quantitative Analysis of protein

Preparation of biuret reagent - Copper Sulphate (1.50 gm) was dissolved in 250 ml of water and sodium potassium tartarate (4.5 gm) and potassium iodide (2.5 gm) were added to it. To this solution, 6 M, 50 ml sodium hydroxide was added and the volume were adjusted to 500 ml with water.

Biuret assay – The solutions were prepared for assay as per quantity, specified in Table No 1. The volume was made up to 10 ml with

water. These solutions were analyzed in a UV-Visible spectrophotometer at 624 nm wavelength. Using same protocol, quantitative analysis of soya protein of unknown concentration may be performed.

Preparation of Buffer solution - Dipotassium hydrogen phosphate (1.452 gm), sodium dihydrogen phosphate (7.601 gm) and sodium chloride (4.8 gm) were dissolved in sufficient water to produce 1 litre of solution.

Electrophoresis - Sufficient amount of buffer solution was poured in the compartment. Whatman filter paper No.1 was cut into strips having a length of 35 cm and width of 5 cm. A spot of sample was applied on a strip of filter paper and marked with pencil. The strips were dipped in buffer solution in such a way that both ends of the paper were in contact with solution. The electrodes were adjoined to compartments; current was allowed to flow (3.2 volts/cm) and electrophoresis was performed for 16 to 18 hours. The strips of filter paper were dried at 100-110°C for 30 min, spots were observed by spraying ninhydrin reagent and their distances were recorded.

Standard amino acid solutions were similarly run on Whatman filter paper and Rf values were recorded.

Simulated Intestinal Fluid - Monobasic potassium phosphate (6.8 gm) was dissolved in 250 ml of water; 77 ml of 0.2 N Sodium hydroxide and 500 ml of hydrochloric acid

were added. To this solution, 10 gm of pancreatin was added and pH was adjusted to 6.8 by adding 0.2 N sodium hydroxide or 0.2 N hydrochloric acid. The volume was adjusted to 1000 ml with water.

Simulated Gastric Fluid - Sodium chloride (2 gm) and pepsin (3.2 gm) were added to 7 ml of hydrochloric acid. The pH was adjusted to 1.2 and the volume was made up to 1000 ml.

Stability Study of Soya Protein in SGF and SIF - The stability study of soya proteins was carried out in SGF and SIF over a period of time. Its stability was measured at different intervals of time (as in minutes), 0 min, 5 min, 10 min, 15 min, 30 min, 60 min. and in hours at 0 hour, 1 hour, 2 hour, 3 hour, and 4 hour. The content of soy protein present in simulated gastric fluid and simulated intestinal fluid was quantified by using Biuret test as specified above.

3. RESULTS AND DISCUSSION

Human diet comprises of carbohydrates, fats, proteins, vitamins, minerals and water. Each of it has its own significance in our body hence it becomes necessary to provide it the required amount. Out of these components, proteins are important structural and functional units of human body. Proteins have diverse varieties of biological actions in human body. These proteins are important biochemical catalysts in the human body and study of these has prime importance in scientific research.

These soya proteins are important regulators in pathogenesis of cancer, osteoporosis and menopausal disorders in females. The soya proteins are hydrolyzed in human body in the gastrointestinal tract and may be absorbed along the GIT at different rates. These proteins are hydrolyzed by peptidases and proteases in GIT. Hence it becomes necessary to study stability of these proteins in biological fluids like GI fluids. To carry out this study, it becomes necessary to use animal model and hence it becomes necessary to carry out stability study of these proteins in *in-vitro* protocols.

3.1. Extraction of soyabean proteins

All the diet used for Soya proteins have their iso-electric pH value on the acidic side, hence these are extracted using sodium bicarbonate solvent. Thereafter this extract was treated with chloroform so as to remove fatty acids and other hydrophobic materials in extract. Thereafter the aqueous layer was partitioned using a dialysis membrane of 10000 kD using TRIS buffer solution. The proteins diffuse across the dialysis membrane into TRIS buffer solution. The proteins in TRIS buffer were thereafter used for further analysis.

Quantitative analysis of soya protein

The soya proteins are quantifiable by Biuret assay protocol and it has been found effective for accurate and precise quantitative estimation of soya proteins.

Regression line equation was used for determining unknown concentration of soya proteins and calibration curve of isolated protein shown in Fig. 1.

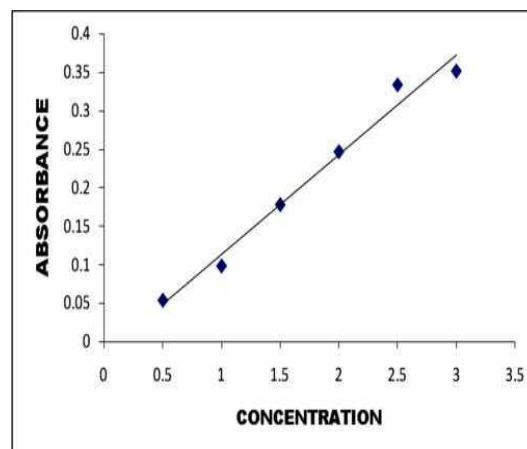


Fig. 1: Calibration Curve of Isolated Soya Protein by Biuret Assay

Table 1: Biuret assay method

S.No.	BSA conc (mg/ml)	BSA Stock (ml)	Biuret reagent (ml)
1	0	0	2
2	1	0.1	2
3	2	0.2	2
4	3	0.3	2
5	4	0.4	2
6	5	0.5	2
7	Sample 1 ml	-	2

Table 2: Quantitative analysis of soya protein by Biuret test

S.No.	Test Solution (ml)	Absorbance	Equation of line for Quantitative Estimation
1.	1	0.053	$\text{Absorbance} = \text{slope} \times \text{concentration} + \text{Intercept}$ $\text{Absorbance} = 0.064914 \times \text{Concentration} + (-0.01687)$
2.	2	0.098	
3.	3	0.178	
4.	4	0.247	
5.	5	0.334	
6.	6	0.352	

From literature survey, it has been found that following amino acids are present in soya proteins. Hence using standard amino acids as markers, the distance travelled by each amino acid was determined and sample solution of soya proteins was also processed for electrophoresis by same method. Thus it has been found that extracted soya proteins contain same amino acids as reported in literature. Arginine, Histidine, Lysine, Tyrosine, Tryptophan, Phenylalanine, Cystine,

Methionine, Threonine, Leucine, Isoleucine, Valine, Glutamic acid, Aspartic acid

After literature review, it has been found that most of *in-vitro* assay protocols have made use of SGF and SIF, official in U.S.P. and results of these *in-vitro* assays matches with *in-vivo* studies. Hence in this protocol, we have made use of SGF and SIF, official in U.S.P. The stability study of soya proteins was carried out in SGF and SIF over a period of time specified in following table.

Table 3: Stability study of soya protein in SGF and SIF

Stability in SGF and SIF		Stability in SGF and SIF	
Time (min)	Percent concentration of soya protein	Time (hour)	Percent concentration of soya protein
0	100	0	100
5	91	1	96
10	72	2	67
15	59	3	61
30	43	4	63
60	38		

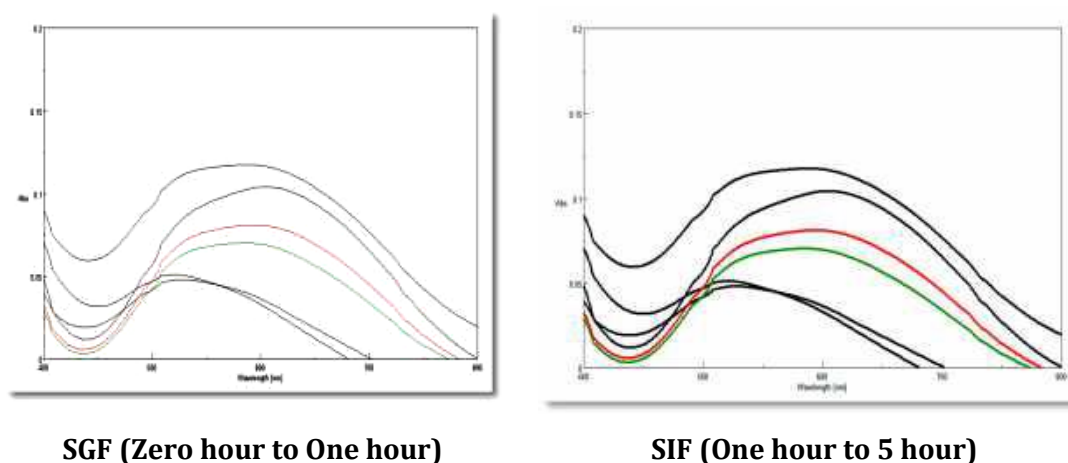


Fig. 2: Overlain Spectrum of Soya Protein Stability Studies

It has been observed that soya proteins are stable in SGF up to 5 minutes and thereafter these will be hydrolyzed and hence found unstable as indicated by their decreasing concentration with increase in time in SGF as well as in overlain spectra of soya proteins concentration over the period of time. The concentration of proteins is determined by Biuret assay protocol specified above.

Similarly soya proteins were stable in SIF up to 1 hour and unstable thereafter as indicated in table no. 3 as well as in overlain spectra of soya proteins concentration over the period of time in the Fig. 2

4. CONCLUSION

Soyabean is an important dietary component which contains abundant amount of proteins or amino acids required by human body for normal growth and maintenance. In this project, isolation of soya proteins was carried out by extraction with 0.01 N sodium

bicarbonate and thereafter homogenization, centrifugation and dialysis of extract against Tris buffered saline solution. These proteins were characterized qualitatively by electrophoresis and quantitatively by Biuret assay method. Biuret assay method is an effective method to quantify amount of protein in given sample. Amino acids present in the isolated protein were identified and quantified using standard or marker amino acids. The stability study of isolated soya proteins was carried out in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), prepared according to protocol specified in USP. These proteins were quantified by Biuret assay method after respective time interval in above specified fluids. The soya proteins were found getting hydrolyze in SGF after 5 minutes and in SIF within one hour. Thus *in-vitro* stability study of proteins in simulated fluids is an effective

approach and alternative to animal model for bio-stability assessment.

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for providing required facilities to carry out this research work.

ABBREVIATIONS:

SGF Simulated Gastric Fluid; SIF, Simulated Intestinal Fluid; GIT, Gastro-Intestinal Tract.

oybean-and-nutrition/soybean-food-or-remedy.

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