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# Formulation and Characterization of Soya Protein Loaded Dosage For Protein Deficiency

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## ABSTRACT

Nutrition has been clearly identified as a key factor in Human development, not only as a conditioning factor for health but also as a determinant of quality of throughout the life cycle, malnutrition affect not only mortality and morbidity figures, but also growth protein deficiency also affect growth and wellbeing of human Present study taken for treatment of protein deficiencies by isolating proteins from soya beans and formulate them in suitable dosage form.

**Keywords:** Soya beans, Soy protein isolation.

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# INTRODUCTION

Protein S deficiency is a disorder associated with increased risk of venous thrombosis.[1] Protein S, a vitamin K-dependent physiological anticoagulant, acts as a nonenzymatic cofactor to activate protein C in the degradation of factor Va and factor VIIIa.[2] Decreased (antigen) levels or impaired function of protein S leads to decreased degradation of factor Va and factor VIIIa and an increased propensity to venous thrombosis. In terms of treatment for protein deficiency the following are consistent with the management (and administration of) individuals with this condition ( it should be noted that the prognosis for inherited homozygote is usually in line with a higher incidence of thrombosis for the affected individual[1].The cause of protein S deficiency it can be in inherited via autosomaldominance. A mutation in the PROS1gene triggers the condition. The cytogenetic location of the gene in question is chromosome 3, specifically 3q11.1[34] Protein S deficiency can also be acquired due to vitamin K deficiency, treatment with warfarin, liver disease, and acute thrombosis (antiphospholipid antibodies may also be a cause as well)[1]

Treatment for protein S deficiency the following are consistent with the management (and administration of) individuals with this condition (it should be noted that the prognosis for inheritedhomozygotes is usually in line with a higher incidence of thrombosis for the affected individual[1]

Soybeans are an abundant and relatively inexpensive source of proteins that are widely recognized for their high nutritional value and excellent functional properties. Functional properties of soyproteins have been exploited in a multitude of applications (for example,

solubility in beverages, foaming in whipped toppings, and emulsification in processed meat) resulting in an ever-increasing demand for soy protein ingredients with improved processing and functional characteristics.[5] Alternate processes in use for soy protein isolation demand industrial ultrafiltration membranes[6], Soy protein isolation process using swellable gels[7], Isolation of soybean protein p34 from oil bodies using hydrophobic interaction chromatography[5], Effect of extraction pH and temperature on isoflavone and saponin partitioning and profile during soy protein isolate production[8], Composition and functional properties of soy protein isolates prepared using alternative defatting and extraction.

# MATERIALS AND METHOD

## Materials

Soy beans were obtained from local market. All other chemicals and reagents were supplied by S.D. lab chemical centre Mumbai -400 002 (India).

**Method**

**Isolation of protein from soya beans**

Three trials I, II, III were taken by using following procedure (Table 1). 10 gm of dried soya flakes free from foreign matter were taken, finely powdered and extracted with 8ml (trial I)/ 40ml (trial II) / 5 ml (trial III) of 0.05N NaOH at 30°C for 15-16 minute and stirred at speed of 600 rpm. After extraction the aliquot was immediately neutralized with 0.1N Hydrochloric acid to pH 8, pH was adjusted with stirring and froth formation was avoided. Then, aliquot was centrifuged at 10,000 RPM for 2 min. and supernatant liquid was removed. The protein content of aliquot was analyzed by using Biuret method for protein determination. The pH of supernatant liquid was adjusted to 5.3 with 1N hydrochloric acid slowly under stirring. The insolubilized protein curd which precipitated out was separated by centrifugation. The protein content of supernatant was analysed by using Biuret method for protein determination. Protein precipitate was dried at 50-60°C by hot air oven drying.[9]

**Table 1: Name of Chemicals Used and Their Quantity in Trial I, II and III[9]**

| Chemicals Used | Trial I | Trial II | Trial III |
|---|---|---|---|
| Soya beans: 0.05N NaOH Ratio | 1:8 | 1:40 | 1:5 |
| Soya beans(grams) | 10 | 10 | 10 |
| 0.05N NaOH (ml) | 80 | 400 | 50 |
| 0.1N HCL (ml) | 22 | 98 | 14 |
| 1N HCL (ml) | 2 | 6 | 1 |

**Characterization of isolated soya protein**

**Test for Protein Content:**

| PRESENT TEST: | ABSENT TEST: |
|---|---|
| 1) Biuretic Test | 1)Molish Test |
| 2) Ninhydrin Test | 2)Heller Test |
| | 3)Tollen's Phloroglucinol Test |
| | 4)Selwinoff's Test |

**1) Biuret test for protein determination**

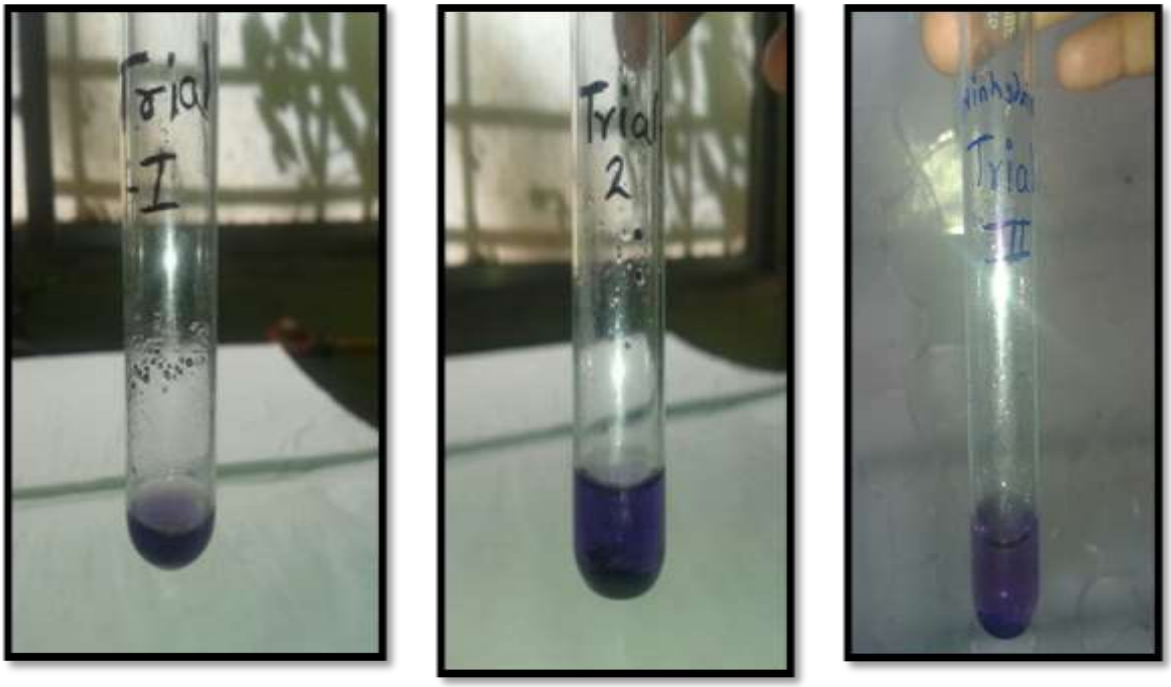
Four test tubes were taken and were cleaned and dried. Test tubes were marked as 1) Blank, 2) Standard I, 3) Standard II and 4) Test. Standard I was prepared by dissolving 4gm of gelatin in 100 ml distilled water. Standard II was prepared by dissolving 8gm of gelatin in 100ml of distilled water. Both standard solutions were prepared with aid of gentle heat with shaking. In blank 0.5ml

distilled water was added. Then, 4ml of Biuret reagent was added in each of the tubes by pipette. The solutions were mixed by stirring and were kept in water bath maintained at temperature of 370C for 10min. Intensity of resulting solutions of test, standards and blank was measured at 100%T at 520-540nm(green filter).[9]


**Trial I** **Trial II** **Trial III**

**Figure 1: Photographs of Biuret Tests**

**Ninhydrin Test**




**Trial I** **Trial II** **Trial III**

**Figure 2: Photographs of Ninhydrin Tests**

**Table 2: Percentage Yield of Protein in Trial I, II and III**

| Trial | Percentage Yield of Protein |
|---|---|
| I | 25 |
| II | 24 |
| III | 30 |

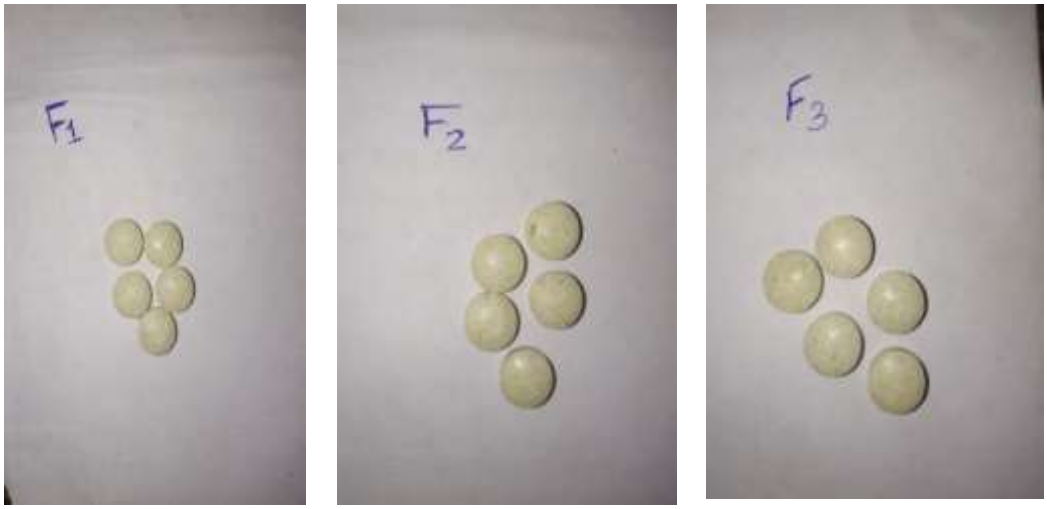
**Preparation of soya protein loaded tablet**

Formulation of protein tablet was carry out in three batches F1,F2,F3 on a rotary press by using direct compression technique by keeping the concentration of protein (200mg) constant and changing the concentration of various additives Such as Binder, diluents, glident as mention in table below.

**Formula:-**

**Table 3: Chemical use in tablet preparation:-**

| Ingredient | F1 | F2 | F3 |
|---|---|---|---|
| Protein | 200 | 200 | 200 |
| Avicel 101 | 100 | 150 | 200 |
| Lactose | 200 | 150 | 100 |
| Talc | 2 | 2 | 2 |
| Magnesium stearate | 2 | 2 | 2 |




**Physicochemical evaluation of soya protein loaded matrix**

**Angle of Repose:**

Angle of repose was determined using funnel method. The blend was poured through funnel that can be raised vertically until a maximum cone height (h) was obtained. Radius of the heap (r) was measured and angle of repose was calculated using the formula.[10]

Tan ɵ= h/r

ɵ= tan-1h/r

Where, θ is the angle of repose, h is height of pile; r is radius of the base of pile.

**Bulk Density:**

Apparent bulk density (Db) was determined by pouring the blend into a graduated cylinder. The bulk volume (Vb) and weight of powder (M) was determined. The bulk density was calculated using the formula[10]

Db = M/Vb

**Tapped Density:**

The measuring cylinder containing known mass of blend was tapped for a fixed time. The minimum volume (Vt) occupied in the cylinder and weight (M) of the blend was measured. The tapped density (Dt) was calculated using the following formula

Dt= M/Vt

**Carr's Compressibility Index:**

The simplest way of measurement of free flow of powder is compressibility, an indication of the ease with which a material can be induced to flow is given by compressibility. Compressibility index of the granules which is calculated by using the following formula

I = Db-Dt/Dbx 100

**Hausner Ratio:**

Hausner ratio is an indirect index of ease of powder flow. It is calculated by the following formula

Hausner ratio= Dt/Db

Where Dt is tapped density and Dd is bulk density.

Lower Haunser ratio (< 1.25) indicates better flow properties than higher ones6 (>1.25).

**Hardness test:**

Tablets require a certain amount of strength, or hardness and resistance to friability, to withstand mechanical shocks of handling in manufacture, packaging and shipping. The hardness of the tablets was determined using Monsanto Hardness tester. It is expressed in $Kg/cm^2$. Three tablets were randomly picked from each formulation and the mean and standard deviation values were calculated.[10]

**Friability test:**

It is the phenomenon whereby tablet surfaces are damaged and/or show evidence of lamination or breakage when subjected to mechanical shock or attrition. The friability of tablets was determined by using Roche friabilator. It is expressed in percentage (%). Four tablets were initially weighed (W initial) and transferred into friabilator. The friabilator was operated at 25 rpm for 4 minutes or

run up to 100 revolutions. The tablets were weighed again (W final). The percentage friability was then calculated by,

F=W initial - W final / W initial×100

% Friability of the tablets less than 1% is considered acceptable.

## RESULTS AND DISCUSSION

**Biuret test for protein determination:-**

| Trial | Protein content |
|---|---|
| I | 0.990 |
| II | 1.05 |
| III | 0.325 |
| STD. I | 2.5 |
| STD.II | 2.82 |

**Angle of Repose:-**

| Batch | Angle of Repose |
|---|---|
| F1 | 31.5° |
| F2 | 30.46° |
| F3 | 30.76° |

**Bulk Density& Tapped Density:-**

| Batch | Bulk Density | Tapped Density |
|---|---|---|
| F1 | 7 | 6.5 |
| F2 | 6.8 | 6.1 |
| F3 | 6.5 | 6.1 |

**Carr's Compressibility Index:-**

| Batch | Carr's Compressibility Index |
|---|---|
| F1 | 7.69 |
| F2 | 10.29 |
| F3 | 6.15 |

**Hausner Ratio:**

| Batch | Hausner Ratio |
|---|---|
| F1 | 0.92 |
| F2 | 0.89 |
| F3 | 0.93 |

**Hardness test:-**

| Batch | Hardness test |
|---|---|
| F1 | 1.5 |
| F2 | 2 |
| F3 | 2 |

**Friability test:-**

| Batch F1 | | Batch F2 | | Batch F3 | |
|---|---|---|---|---|---|
| Initial Weight | Final Weight | Initial Weight | Final Weight | Initial Weight | Final Weight |
| 500 | 500 | 500 | 500 | 510 | 500 |

| | | | | | |
|---|---|---|---|---|---|
| 510 | 490 | 510 | 500 | 500 | 490 |
| 500 | 500 | 500 | 495 | 500 | 490 |
| 500 | 500 | 500 | 500 | 510 | 500 |
| 500 | 495 | 500 | 500 | 490 | 490 |

## RESULTS AND DISCUSSION

**Isolation of protein from soya beans**

Three trials were conducted and the protein yield was calculated (Table). Trail II proved to offer best yield. In this procedure time required for extraction of protein from soya beans was reduced. This improved total yield of protein and preserved quality of protein. Resulting protein showed less beany taste. Reported methods for extraction of soy protein demonstrate about 22-28 % soy proteins yield. Soy bean contain 35-40 % protein. Current method gives 24% yield even after subjecting the soya bean to alkaline condition only for 15 minutes under stirring. This gives good yield and hence seems to be more suitable for soy protein extraction.

**Characterization of isolated soya protein**

Various test were conducted, from which Ninhydrin test & Biuret test was found to be positive, As this test are important for protein detection. Biuret test of supernatant liquid was performed from which TRIAL II got the highest percentage of protein in it.

**Preparation of soya protein loaded matrix.**

Formulation of tablet containing 200mg of protein and Avicel102 200mg and lactose 100mg was optimized formulation having proper hardness, friability and free flowing nature of its powder.F1,F2 formulation were near to the readings of F3 but the friability and angle of repose showed poor results than F3.

**Physicochemical evaluation of soya protein loaded matrix**

**Angle of repose:-**

The angle of repose of optimized batch that's F2, according to standard was excellent.

**Bulk Density & Tap Density:-**

The bulk density, Tap density, Hausner ratio & Carr's Compressibility Index of optimized batch that's F2, according to standard was very good.

**Hardness test:-**

The hardness of batch F2 was found in optimized range as per standard.

**Friability test:-**

The friability (F2) of all tablet formulations was in acceptable range of less than 1%, ranging i.e. 0.6 ± 0.01 % which indicates that the tablets have the ability to withstand shocks which may be occurred during transport

## CONCLUSION

It can be concluded that the proposed method of extraction for soy protein is simpler and gives a better quality protein with good yield at laboratory scale. This is due to reduced exposure time to highly alkaline conditions. Thus, proteins were protected from hydrolysis.[9] It can also be concluded that the formulated tablet F2 was optimized as it had proper hardness, friability, also the powder of this formulation showed good flow ability which was confirmed by determination of angle of repose, also it had proper bulk and tapped density. Thus, it was considered to be optimized.

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