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Preparation and Characterization of Soya Protein Loaded Capsules

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ABSTRACT

Every cell, tissue and organ in your body contains the micronutrient protein, which provides your body with energy. The digestive process breaks down protein in food into amino acid that repair and replenish the body. Protein helps to build muscles, produce new cells, regulate hormones and enzymes, heal wounds and promote immune function. Low dietary protein is most common in developing countries due to inadequate access to protein-rich foods. However, it can also affect people in developing countries who make poor dietary selections. Insufficient dietary protein can result in many negative side effects. So to avoid such negative effect we taken project to isolate protein and formulate into capsule.

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]Proteins, 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]

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 in solubilised 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'sPhloroglucinol 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]

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 capsule

Formulation of protein capsule was carried out by isolating protein from soya beans and filling the proteins in capsule.

Physicochemical evaluation of soya protein loaded Capsule

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).

Uniformity of weight :-

5 capsules are selected randomly. One capsule is weight and the capsule is opened to remove the content, and the empty capsule is weight again. Then, the weight content inside the shells is determined. The procedure is repeated with other 4 capsules. The average net weight is determined from the sum of individuals' net weight. The percentage deviation from the average net weight for each capsule is determined

Uniformity of mass

Weigh 20 intact capsules individually, and calculate the average mass. The mass of each capsule should be within ±10% of the average mass. If all the capsules do not fall within these limits, weigh the 20 capsules again, taking care to preserve the identity of each capsule, and remove the contents as completely as possible. For soft gelatin capsules, wash the shell with ether or some other suitable solvent and allow it to stand until the odour of the solvent is no longer perceptible. Other means, such as a jet of compressed air, may be used to remove the contents. Weigh the emptied shells individually and calculate for each capsule the net mass of its contents by subtracting the mass of the shell from the gross mass. Determine the average net content from the sum of the individual net masses. Then determine the difference between each individual net content and the average net content. Deviation of individual net mass from the average net mass should not exceed the limits given below.

Disintegration test

Place one dosage unit in each of the six tubes of the basket and if specified add a disc. Operate the apparatus using water as the immersion fluid unless another liquid is specified and maintain its temperature at 35–39 °C. At the end of the specified time lift the basket from the fluid and observe the dosage units: all of the dosage units have disintegrated completely. If one or two dosage units fail to disintegrate repeat the test on 12 additional dosage units. The requirements of the test are met if not less than 16 of the 18 dosage units tested are disintegrated.

RESULTS AND DISCUSSION

Biuret test for protein determination:-

TRIAL	Protein content
I	0.990
II	1.12
III	0.425
STD. I	2.5
STD.II	2.62

Angle of Repose:-

Sample	Angle of Repose
Protein powder	30.46°

Bulk Density& Tapped Density:-

BATCH	Bulk Density	Tapped Density
Protein powder	6.8	6.1

Carr's Compressibility Index:-

BATCH	Carr's Compressibility Index
Protein powder	10.29

Hausner Ratio:

BATCH	Hausner Ratio
Protein powder	0.89

Uniformity of Weight

Capsule no.	Weight of protein (gm)
1	0.49
2	0.50
3	0.50
4	0.48
5	0.49

Uniformity of mass

Capsule no	Weight of empty capsule shell (gm)	Weight of shell along with protein powder(gm)	Weight of active drug (gm)
1	0.09	0.580	0.49
2	0.09	0.590	0.50
3	0.110	0.610	0.50
4	0.09	0.570	0.48
5	0.09	0.580	0.49

Disintegration test

Capsule no.	Time to disintegrate (min)
1	1.25
2	1.30
3	1.25
4	1.26
5	1.28
6	1.32

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.

Physicochemical evaluation of soya protein

Angle of repose:-

The angle of repose, according to standard was excellent.

Bulk Density & Tap Density:-

The bulk density, Tap density, Hausner ratio & Carr's Compressibility Index of the capsule complies with the limits of standard .

Uniformity of weight :-

The test for uniformity of weight showed result complying standard values.

Uniformity of mass

The test for uniformity of mass showed result complying standard values.

Disintegration time:-

The Disintegration test proves that the all the capsules had been disintegrated and within 1.5 min.

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 capsule was optimized as it had higher protein content than any other dosage form

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