

Extraction of Collagen Protein by Acid Soluble Collagen from Soya Seeds

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ABSTRACT

Considering the two collagen rich foods and extracted out the collagen protein from it by the method of acid soluble collagen (ASC). Collagen is naturally occurring fibrous protein which is important in maintaining the health and vitality of connective tissues. They work with elastin, another structural protein to maintain connective tissues elasticity. They may get benefit from the two amino acids lysine and proline and the presence of collagen promoting nutrient such as vitamin C. In the present study determination was done by ASC in which the sample was dissolved in 30 ml of 0.1N NaOH and filtration was done. After that the supernatant took as acid soluble followed by precipitation with 2.6M NaCl involving centrifugation at 38,400rpm for about 20 minutes and the residues were separated. At last, wet and dried separated residues were taken for protein determination by kjeldahl's method. From this study it was concluded that the dry and wet percentage of protein is more than the total soy protein which means that the extracted protein was collagen.

Keywords: Acid soluble, Collagen, Protein, Sources.

INTRODUCTION

Collagen is the most abundant animal protein polymer almost 30% of total protein in animal body is collagen. It is major fibrous protein in connective tissues. Collagen has wide range of applications in biomedical, cosmetics, leather and foods, pharmaceutical industries¹.

Collagen is regarded as one of the most useful biomaterial mainly due to its non- toxicity, immunological properties and biocompatibility².

It is a species of legume native to East Asia, widely grown for its edible bean which has numerous uses. The plant is classed as an oilseed rather than a pulse by the UNFAO (United Nation Food & Agricultural Organization). Fat free soyabean meal is a significant and cheap source of protein for animal feeds and many packaged meals; soy vegetable oil is another product of processing the soyabean crop. Soyabean produce significantly more protein per acre than most other uses of land.

Nutrela contains all essential amino acids, making it rich in proteins. It is light and easy to digest and it is excellent for growing children, expectant mothers and sportsmen. As per USFDA, consumption of 25g of soya protein daily may help reduce the risk of coronary heart diseases. It is low in fat and keeps us energetic and active throughout the day, making it perfect for those who are on weight management programme.

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Soybean is hailed as the most protective bean. Soy contains 40 percent protein. It has the highest protein content amongst plant products. "Soy protein" refers to the protein found in soya beans³. As animal protein contains all the essential amino acids, lacking in pulse protein, soy is often used to replace the animal proteins in an individual's diet. It is the only food that contains all the eight essential amino acids. Soya beans are processed to various soya products namely soy flour, soy milk, cottage cheese like tofu, fermented products like tempeh and miso. It is a complete food and are rich in vitamins and minerals including folate, potassium and in some cases, fiber.

It may reduce the risk of heart disease. Most soy foods are also low in saturated and trans fats, one reason why the American Heart Association has recognized soy foods role in an overall heart healthy diet.

It may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems and alleviate hot flashes associated with menopause.

Soy isoflavones, a factor in helping to prevent bone loss. The soy isoflavones genistein seems to inhibit bone breakdown and may have similar effects than estrogens in maintaining bone tissue. Most symptoms of menopause are caused by low estrogen levels. Estrogen plays a role in body temperature control.

MATERIALS AND METHODS

Determination of Moisture

For this, drying oven method was used. The sample was taken and crushed into powder form. After that, the moisture dish was taken, washed, rinsed and dried it in a drying oven. After drying, the moisture dish was kept in the dessicator to maintain the temperature. The empty weight of the moisture dish was taken. 5g of sample was weighed in the moisture dish. The moisture dish was kept at 130 degrees for 2h (as mentioned in the IS) in the oven. After two hours, the moisture dish was kept out from the oven and put into the dessicator. Now, after 25 minutes the readings were noted. Again the moisture dish was kept in hot air oven for 1h at the same temperature. The process was continued till the weight becomes constant.

Determination of Ash

For ash determination, the silica dish was taken, washed, rinsed and dried it. The empty weight of the silica dish was taken. 5g of sample in the silica dish was weighed and kept on hot plate till the fumes did not appear. After that, the dish was kept in the muffle furnace for ignition at a temperature not more than 550 degrees for about 5-6 hours. Cooled in a dessicator and weighed. The process was repeated till constant.

Determination of Fat

For the determination of fat, round flat bottom flask was taken, washed, rinsed and dried it. The empty weight of the flask was taken. 10g of sample in the thimble was weighed and plugged with cotton. The thimble was put in the sifon tube and then petroleum or diethyl ether on the sample was poured. The volume was made hanging up to 250ml. For 16h it was allowed to run in the water bath for reflux. The residue were there, then filtered through sodium sulfate wet with petroleum ether. The sample was prepared and washings were given. The sample was let to evaporate in the water bath above temperature 90 degrees. It was made constant.

Determination of Protein

It is defined as the amount of nitrogen experimentally found and multiplied by an appropriate conversion factor. The sample is oxidized in the presence of sulphuric acid and nitrogenous compounds are converted into ammonium sulphate. Mercury is added to the digestion mixture as a catalyst and alkali sulphate as a boiling-point-elevator. Ammonia is liberated by adding an excess of alkali and is quantitatively distilled into a measured volume of standard hydrochloric or sulphuric acid. The acid not neutralized by ammonia is back titrated with standard alkali.

For Digestion: 500-800 ml kjeldahl flasks was used. Digestion over a heating device was conducted and adjusted to bring 250ml water at 25 degrees to rolling boil at approximately 5 minutes. The heaters were tested, pre-heated for 10 minutes, if gas, or for 30 minutes if electric. 3-4 glass beads were added to prevent superheating.

For Distillation: The flask was fitted with a rubber stopper through which the lower end of an efficient scrubber trap or bulb was passed to prevent mechanical carryover of alkali during distillation. The upper

end of the trap was connected to a condenser by rubber or glass tubing. The trap outlet was immersed of the condenser in such a way as to ensure complete absorption of ammonia distilled over into acid in an 500ml Erlenmeyer flask.

The protein estimation was done by ASC method using kjeldahl's apparatus. Accurately 0.8653g sample into the digestion flask was weighed. 10g sodium sulfate and 0.5g copper sulphate in the kjeldahl flask were added. 25ml of conc. Sulphuric acid was added and kept for the digestion on the heating mantle. A small amount of silica was added to reduce foaming. The solution vigorously boiled until it becomes clear and then kept it to cool. After cooling, about 200ml of distilled water was added and few granules of silica or glass beads were added to prevent bumping and then sodium hydroxide solution was added to the connected flask to distillation condenser. All ammonia was heated until it has distilled over. The receiver was lowered before stopping distillation and tip of condenser was washed with the distilled water. The excess acid with standard 0.1N sodium hydroxide using methyl red as indicator was back titrated. The blank was taken for the determination using all the reagents. Titrated till the color changes to golden yellow.

Determination by ASC (Acid Soluble Collagen):

For this, the sample was taken and dissolved it in 30 volumes of 0.1N NaOH solution. After dissolving, filtration was done. Now, the supernatant as acid soluble (0.5M CH₃COOH followed by precipitation with 2.6M NaCl) was taken. After that centrifugation was done at 38,400 rpm for about 15-20 minutes. The residues and the supernatant were separated. The residues then further was taken for the dry yield (moisture constant) and wet yield and the supernatant was discarded. The wet residue then further be used for the protein determination and the dried part after constant weight by air dry oven method also used for protein determination⁴.

RESULTS AND DISCUSSION

Collagen for biomedical applications is mainly isolated from animal tissues (bovine or porcine skin and bovine or equine Achilles tendons). Fish scales are composed of collagen covered with calcium salts. In the present study we report the preparation of collagen from fish scales for potential cosmetics, pharmaceuticals and implant applications. It was the first time that the species *Esox Lucius* used as a source of collagen.

There are major methods of collagen extraction. These are salt solubilized collagen, acid solubilized collagen and pepsin- solubilized collagen. Table 1 showed the moisture estimation and Table 2 showed Ash determination.

Table 1 Moisture Determination

Empty weight	Sample weight before drying	Sample weight after drying	Moisture (percent by weight) = 100 (w1-w2)/w1-w % Moisture
31.0649g	5.0172g	35.6610g	8.39%

W1: weight gram of dish with material before drying

W2: weight gram of dish with material after drying

W: weight gram of empty moisture dish

Table 2 Ash Determination

Empty weight of silica dish	Sample weight before ash	Weight of ash after dry	Total ash (percent by weight) = 100 (w2-w)/(w1-w) % Total Ash
33.2896g	4.1530g	33.3962g	0.273%

W1: weight gram of silica dish with sample

W2: weight gram of dish with ash

W: weight of empty dish

Table 3 and Table 4 showed Fat determined and protein determination.

Table 3 Fat Determination

Empty weight of round bottom flask	Sample weight in thimble	Weight after fat extraction (constant)	% Fat = weight of residue/sample weight * 100% Fat
138.8686g	10.2038g	138.8922g	0.2305%

Table 4 Protein Determination

A	B	C	D	Protein percent by mass
volume in ml 0.1n acid measured for main distillation	volume in ml 0.1N alkali used for back titrating A	volume in ml 0.1N acid measured for blank distillation	volume in ml 0.1N alkali used for back titrating C	N* 100*conversion factor/W= 49.18%

$$\text{Calculation} = (55.1 - 6.6) * 6.25 * 1.401 * 0.1002 * 100 / 1000 * 0.1 * 0.8653 = 49.18\%$$

$$\text{Nitrogen content (N) in gram} = (A - B) - (C - D) * 0.0014$$

Where, A = volume in ml 0.1n acid measured for main distillation

B = volume in ml 0.1N alkali used for back titrating A

C = volume in ml 0.1N acid measured for blank distillation

D = volume in ml 0.1N alkali used for back titrating C

Protein percent by mass = N * 100 * conversion factor / W

Where, N = mass of nitrogen in gram of original sample

W = mass of sample in gram

ASC

Dry yield:

Empty weight of moisture dish = 20.7781g

Sample weight = 5.0868g

Dry weight (constant after drying) = 21.1456g

Wet yield:

Empty weight of moisture dish = 20.7781g

Sample weight = 5.0868g

Wet weight = 24.3565g

Soya Dry Protein:

Sample weight = 0.3147g

Titre volume = 32.2ml

Soya Wet Protein:

Sample weight = 0.5g

Titre volume = 24.7ml

- Dry yield percent = dry weight – empty weight/sample weight
= $21.1456 - 20.7781 / 5.0868 * 100$
= 7.22%
- Wet yield percent = wet weight – empty weight/ sample weight
= $24.3565 - 20.7781 / 5.0868 * 100$
= 70.34%
- Soya dry protein = (blank – titre volume)*protein factor*nitrogen factor*normality of NaOH*100/1000*actual normality*sample weight
= $(55.1 - 32.2) * 6.25 * 1.401 * 0.1002 * 100 / 1000 * 0.1 * 0.3147$
= 63.84%
- Soya wet protein = (blank – titre volume)*protein factor*nitrogen factor*normality of NaOH*100/1000*actual normality*sample weight
= $(55.1 - 24.7) * 6.25 * 1.401 * 0.1002 * 100 / 1000 * 0.1 * 0.5$
= 53.34%

In this study, only one method was used. It was acid solubilized collagen method extraction of collagen using other different methods may give positive results. Previous studies showed that extraction of collagen using acid-pepsin gives higher yield results of collagen. So in future, doing collagen extraction with three different methods and comparing their results should be recommended.

Second, this study was done at controlled temperature to yield positive results. Uncontrolled temperature may result in denaturing collagen.

Third, only one type of soya bean was used in this study. It was Nutrela. By using different types of soya bean, it would give different results. In addition to that, only 50gm of soya was used. Using larger amount would give larger amount of collagen and would give chances of detecting collagen.

Fourth, in this study, only 200ml of solution of solubilized collagen was obtained by using double layers of filter paper.

The collagen is sensitive to UV radiations of sunlight which can cause some structural changes in them and it is due to the oxidative effects of these radiations on the collagen of the skin.¹¹In previous study, it was reported that soya bean extract found to instigate the collagen synthesis and correct assembly of elastin fibers and also found to provide the complete protection to the dermal extra cellular matrix⁵. Phytochemical investigation was reported earlier in a study⁶. Phytochemicals present in plants can be a potent source of anti-aging.

CONCLUSION

The findings indicate that collagen extracted from soya bean extract has more dry and wet yield as compared to normal soya bean extract. There is an adequate amount of collagen present which can be easily isolated and utilized for various industrial purposes such as cosmetics and pharmaceuticals use with low or no cost. This extracted collagen can have some potential anti-aging effects.

Conflicts of Interest

We declare that we have no conflict of interest.

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