

Formulation and Quality Evaluation of Cottonseed Protein

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Abstract—The “Formulation and Quality Evaluation of Cotton Seed Protein” was carried out to develop a protein-rich plant-based product from cottonseed milk using different coagulants. Cottonseed, an underutilized by-product of the cotton industry, was explored as an alternative protein source due to its high nutritional potential. Cottonseed milk was prepared, bleached using potassium metabisulphite to improve color, and heat treated at 80–90°C to facilitate protein coagulation. Different coagulants such as lemon juice, vinegar, and calcium chloride were evaluated through several experimental trials to optimize protein recovery and product quality. The optimized treatment resulted in the successful formation of cottonseed protein with improved texture and appearance. The final product showed a protein content of 21.3% and ash content of 6.99%, indicating good nutritional quality and mineral content. However, gossypol analysis revealed residual free gossypol levels of 1.32–1.41% and bound gossypol content of 1.88%, indicating the need for further detoxification. Overall, the study demonstrated the potential of cottonseed as a sustainable alternative plant-based protein source, while emphasizing the importance of advanced processing methods to improve product safety for human consumption.

Index Terms—Cottonseed, Cottonseed milk, Plant-based protein, Cottonseed protein, Coagulation, Calcium chloride, Protein extraction, Potassium metabisulphite

I. INTRODUCTION

The increasing demand for sustainable and nutritionally balanced food systems has led to a significant shift toward plant-based protein sources in recent years. Growing awareness regarding environmental sustainability, efficient utilization of natural resources, health benefits, and changing

dietary preferences has encouraged consumers to explore alternatives to conventional animal-based foods. As a result, plant-based protein products have gained considerable attention due to their nutritional value, affordability, functional properties, and suitability for vegan and lactose-intolerant populations. This transition has created a strong need to identify underutilized plant resources that can serve as economical and sustainable protein sources for future food applications.

Among the various plant-derived resources, cottonseed has emerged as a promising yet underexploited source of nutrition. Cottonseed is an abundant by-product of the cotton industry and is traditionally utilized for oil extraction and animal feed purposes. However, it also contains a considerable amount of protein, essential fatty acids, carbohydrates, and minerals, making it a valuable raw material for the development of plant-based food products. In South India, cottonseed milk is traditionally consumed in the form of “Paruthi Paal,” which highlights its cultural acceptance and nutritional significance. The growing interest in alternative proteins has further increased attention toward cottonseed because of its potential to provide a low-cost and sustainable source of plant protein.

Plant-based proteins obtained from sources such as soy, legumes, cereals, nuts, and seeds are increasingly preferred due to their positive impact on health and the environment. Compared to animal protein production, plant-based proteins require fewer natural resources and contribute to lower environmental pollution. In addition, plant-based foods are associated with several health benefits, including improved digestion and suitability for individuals affected by lactose

intolerance and milk allergies. These factors have accelerated the global demand for innovative plant-based products that can provide both nutritional and functional advantages.

Soy protein has been extensively studied and widely used in the preparation of products such as soymilk and tofu because of its high protein content and desirable functional properties. Tofu, a coagulated soy protein product, is considered an important plant-based protein food due to its nutritional quality and versatility. The success of soy-based protein products has encouraged researchers to explore other plant proteins with similar characteristics. In this context, cottonseed protein offers considerable potential because of its balanced nutrient composition and ability to form protein-rich food products.

Recent studies have demonstrated the successful utilization of cottonseed milk in the development of value-added products such as flavored beverages, probiotic drinks, and plant-based ice cream. These findings indicate that cottonseed milk possesses desirable physicochemical and sensory properties suitable for food applications. Its creamy texture, nutritional composition, and functional characteristics make it a promising alternative to conventional dairy ingredients. Despite these advantages, cottonseed protein remains comparatively underutilized in structured food products, creating opportunities for further research and product development.

The present study focuses on the formulation and quality evaluation of cottonseed protein as a plant-based food product. Cottonseed was selected because of its nutritional potential, sustainability, economic availability, and growing relevance in the field of alternative proteins. The study aims to contribute to the development of innovative plant-based protein products while promoting the utilization of underexplored agricultural resources for sustainable food production.

II. MATERIALS AND METHODOLOGY

Materials:

Raw cottonseeds were procured from a local source (Coimbatore) and used for the study. Fresh lemon juice and commercial vinegar (Aw So Yum) were used as natural coagulants. Calcium chloride (Allas posh Flavors) was used as a coagulant, and potassium

metabisulfite (BCCHEM) was used as a bleaching agent during processing.

III. METHODOLOGY

Formulation And Development of Cottonseed Protein
Good quality cottonseeds were selected from a local source in Coimbatore, making sure they were clean and free from impurities like dust and stones. The seeds were then soaked in water for about 12 hours at room temperature (34–36°C). This step helped to soften the seeds and made them easier to grind, which improves the extraction of nutrients, especially protein. After soaking, the excess water was removed, and the seeds were ground with water using a mixer grinder to form a smooth paste. This ensures that the seed material is properly broken down and the milk can be extracted effectively.

The ground mixture was then filtered using a muslin cloth to separate the liquid part, called cottonseed milk, from the solid residue. The extracted milk was collected and heated to a temperature between 70–90°C. Heating is important because it helps the proteins to denature and prepares them for coagulation. It also helps improve the safety of the milk. After heating, coagulants such as lemon juice, vinegar, and calcium chloride were added to the milk while stirring. These coagulants help in converting the milk into curd by causing the proteins to clump together. The mixture was then allowed to settle so that the curd and whey could separate properly. The curd was then filtered and collected using a muslin cloth.

Finally, the curd was gently pressed to remove excess water and to obtain a firm and compact protein product. The final product was then stored at 5°C to maintain its quality and prevent spoilage before further analysis and evaluation. Supporting literature by J. Vijaya Chandiya *et al.* (2025) reported pasteurization of cottonseed milk at 85–90°C, which aligns with the findings of the present study. While lower temperatures (around 70°C) have been reported in other food applications such as ice cream processing, they are insufficient for proper coagulation in cottonseed milk. Therefore, the use of higher temperatures in this study ensured better protein coagulation, reduced microbial load, and improved structural integrity of the final product.

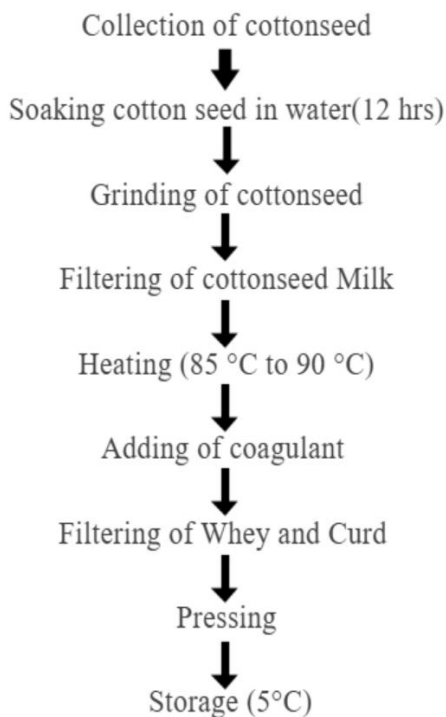


Fig:3.1 Process Flowchart for Formulation of Cottonseed Protein

Product Development Trials

In the initial trial, cottonseed milk was heated to 70°C for 30 seconds and treated with lemon juice as a coagulant. However, the result was a liquid slurry with poor curd formation, indicating that the applied temperature and time were insufficient to induce proper protein denaturation and coagulation. This suggests that cottonseed proteins require higher thermal energy for effective structural modification. Subsequent trial involved increasing the temperature to 80°C and evaluating different concentrations of coagulants, specifically lemon juice (citric acid) 10 ml, 15 ml, and 20 ml added to 115 ml of cottonseed milk. We found that the 15 ml concentration produced the best coagulation and texture. In contrast, the 10 ml addition resulted in incomplete curd formation, while 20 ml made the texture slightly tough. Thus, the second trial identified 15 ml as optimal.

In the third trial, vinegar was used as the coagulant at concentrations of 10 ml, 15 ml, and 20 ml, with the milk heated to 80°C. Among these, 20 ml produced the best coagulation with a firm and well-formed texture. The 10ml and 15 ml concentrations resulted in weaker and less defined curd formation. Hence, 20ml vinegar

was found to be the most effective concentration in this trial. In the fourth trial, acetic acid was used as the coagulant, with the milk maintained at 80°C and the heating time increased to achieve proper coagulation. The samples, including raw cottonseed milk and the final coagulated product, were subjected to gossypol analysis. The gossypol content was found to be 1.23% in the cottonseed milk and 1.36% in the final product. This indicates that gossypol was still present after processing, showing the need for further treatment to reduce its level.

In the fifth trial, only heated cottonseed milk was used for analysis, without adding any coagulant. The milk was heated to 90°C for about 10 minutes and then subjected to gossypol testing. The results showed that the free gossypol content was 1.32% and the bound gossypol content was 1.88%. This indicates that heat treatment alone did not significantly reduce the gossypol content, suggesting the need for additional processing methods. In the sixth trial, calcium chloride was used as the coagulant at 90°C. Different concentrations (0.111 g, 0.222 g, and 0.333 g) were tested to evaluate their effect on protein recovery. It was observed that increasing the concentration up to 0.222 g improved the protein yield, while a further increase to 0.333 g did not show any significant improvement. Therefore, 0.222 g was identified as the optimal concentration for effective coagulation and maximum protein recovery.

In the seventh trial, cottonseed was first bleached using potassium metabisulphite (0.015 g) by soaking for 10 minutes and then washing. The aim of bleaching was to reduce the natural pigments and improve the color of the milk, but only a slight change in colour was observed. Cottonseed milk (100 ml) was then prepared and heated to 90°C, after which calcium chloride (0.222 g) was added as a coagulant. This resulted in proper coagulation and a protein yield of 16.2 g. The bleaching slightly improved the milk quality, and calcium chloride helped in better protein formation. In the last trial, a second stage of bleaching was carried out using a higher amount of potassium metabisulphite (0.025 g), followed by washing, which showed better improvement in color compared to the first stage. Cottonseed milk (200 ml) was extracted from 200 g of seeds and heated to 90°C. Vinegar (40 ml) was then added as a coagulant, resulting in effective coagulation and a higher protein yield of 22.5 g. The improved bleaching along with better

processing conditions helped in obtaining a higher yield. The final sample was sent for laboratory analysis to determine protein, ash, and free gossypol content. Potassium metabisulphite (INS 224) is permitted by FSSAI as a preservative and antioxidant. The permissible sulphite limit for liquid food systems is generally 50–200 ppm (mg/kg). In the present study, 0.025 g KMS was used for 200 g cottonseed, corresponding to approximately 125 ppm, which is within the permissible FSSAI limit

Analysis of Cottonseed Protein

The developed cottonseed protein product was subjected to various analytical tests to evaluate its nutritional quality and safety characteristics. Parameters such as protein content, ash content, pH, and gossypol content were analyzed using standard analytical methods. These analyses were carried out to determine the composition, quality, and suitability of the developed product. The obtained results helped in assessing both the nutritional value and safety aspects of the cottonseed-based protein product.

Protein content

0.7–2.2 g of the sample was taken in a Kjeldahl flask, and mercuric oxide, potassium sulphate, and concentrated sulphuric acid were added. The mixture was first heated gently and then boiled until a clear solution was obtained, with digestion continued for 1–2 hours. After cooling, distilled water was added along with sulphide or thiosulphate solution and a few zinc granules. The solution was then made alkaline using sodium hydroxide, and the liberated ammonia was distilled into a measured volume of standard acid. The excess acid was subsequently titrated with standard sodium hydroxide using methyl red as an indicator, and a blank determination was also performed. Protein content was estimated using the Kjeldahl method as per Bureau of Indian Standards (BIS), IS 7219:1973. The equipment used in this analysis included a Kjeldahl digestion flask, which facilitates the digestion of the sample during nitrogen estimation.

Ash content

5 g of the prepared sample were accurately weighed into a clean, dry silica dish and gently ignited over a burner until completely charred. The dish was then placed in a muffle furnace maintained at $550 \pm 10^\circ\text{C}$ and heated until a grey ash was obtained. After cooling

in a desiccator, the dish was weighed, and the processes of heating, cooling, and weighing were repeated until a constant weight was achieved (difference less than 1 mg). The final constant weight was recorded for ash content determination, following the method prescribed in Bureau of Indian Standards (BIS), IS 12711:1989. The equipment used in this analysis included a muffle furnace, which is used for high-temperature ashing of the sample, and a silica dish, which serves as a heat-resistant container for holding the sample during ignition.

pH

10 g of the sample was weighed and transferred into a beaker, followed by the addition of an adequate quantity of distilled water. The mixture was stirred thoroughly to obtain a uniform slurry. The pH meter was calibrated using standard buffer solutions of pH 4.0, 7.0, and 9.2. The electrode was then immersed into the prepared sample, and the reading was allowed to stabilize before recording the pH value directly from the instrument. This procedure was carried out as per the Food Safety and Standards Authority of India Manual of Methods of Analysis of Foods. The equipment used in this analysis was a pH meter, which accurately measures the hydrogen ion concentration of the sample to determine its pH.

Gossypol content

The ground sample was weighed and transferred into a flask, followed by the addition of a complexing agent solution. The mixture was heated in a boiling water bath for 30 minutes and then cooled. It was subsequently diluted with a hexane–isopropyl alcohol mixture, filtered, and made up to a known volume. An aliquot of the extract was taken in a volumetric flask, and a few drops of hydrochloric acid were added. Iron (III) solution was then introduced, mixed thoroughly, and allowed to stand for about 5 minutes to develop color. Finally, a small amount of water was added, and the solution was made up to volume with the same solvent mixture. The absorbance was measured at 620 nm using a spectrophotometer against a reagent blank. This total gossypol estimation was carried out using a spectrophotometric method based on the reaction of gossypol with a complexing agent followed by iron (III) complex formation, as described by Admasu and Chandravanshi (1984). The analysis was performed using a UV–Visible spectrophotometer, which

measures absorbance at 620 nm, and volumetric flasks, which were used for preparing solutions of accurately known volume.

IV. RESULTS AND DISCUSSION

Several experimental runs were conducted to optimize the conditions for protein extraction by varying temperature, coagulant type, and concentration, with heating carried out under controlled temperature conditions.

Product Development Trials

A series of experimental trials were conducted to optimize the process conditions for the extraction of protein from cottonseed milk. In total, eight trials were carried out by systematically varying key parameters such as the quantity of cottonseed used, volume of extracted milk, temperature, duration of heating, and type and concentration of coagulant. The primary objective of these trials was to identify the most suitable combination of conditions that would result in maximum protein yield with desirable texture and quality. Both natural coagulants (lemon juice and vinegar) and chemical coagulants were evaluated for their efficiency in protein coagulation. Heating temperatures ranging from 70°C to 90°C were applied to study their effect on protein denaturation and aggregation. Each trial was carefully observed and recorded based on the extent of coagulation and yield obtained. The optimized conditions derived from these trials were further used for product evaluation and analysis.

Table.1 Experimental Trials for Cottonseed Milk Protein Coagulation

(without bleaching)

S.No	Parameter	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
1	Cottonseed used for extraction	250g	200g	200g	200g	200g	300 g
2	Cottonseed milk used	500ml	115ml	135ml	300ml	400ml	100 ml
3	Temperature	70°C (30Sec)	80°C (30 Sec)	80°C (30 Sec)	80°C (10min)	90°C (10min)	90°C (10min)
4	Amount of coagulant	30ml	15ml	20ml	40ml	Not added	0.222 g
5	Yield	Not Obtained	10.5g	14.7g	25.6g	Not obtained	14.3 g

Trial 1

Cottonseed milk (500 ml) obtained from 250 g seeds was heated to 70°C for 30 seconds. Lemon juice (30 ml) was added as coagulant. No proper protein formation was observed.



Fig:3.2a



Fig :3.2b

Trial 2

Cottonseed milk (115 ml) from 200 g seeds was heated to 80°C for 30 seconds. Lemon juice was added at different concentrations (10 ml, 15 ml, 20 ml). Optimal coagulation was observed at 15 ml, yielding 10.5 g of protein.



Fig :3.3a



Fig:3.3b

Trial 3

Cottonseed milk (135 ml) from 200 g seeds was heated to 80°C for 30 seconds. Vinegar was used as coagulant at different concentrations (10 ml, 15 ml, 20 ml). Best results were obtained at 20 ml, yielding 14.7 g of protein.



Fig:3.4a



Fig:3.4b

Trial 4

Cottonseed milk (300 ml) from 200 g seeds was heated at 80°C for 10 minutes using an induction stove. Lemon juice (40 ml) was added as coagulant, resulting in improved protein formation with a yield of 25.6 g. The milk and protein samples were collected and sent for gossypol analysis.

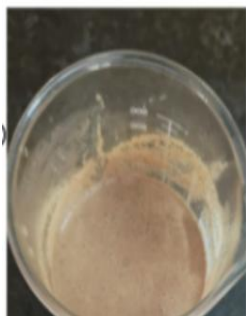


Fig:3.5a



Fig:3.5b

Trial 5

Cottonseed milk (400 ml) from 200g seeds was heated at 90°C for 10 minutes without addition of coagulant. The heated milk was directly submitted for gossypol analysis to study the effect of heat treatment alone. Based on the trial results, higher temperature and optimized coagulant concentration were found to enhance protein coagulation and yield.



Fig :3.6a



Fig:3.6b

Trial 6

Cottonseed milk (100 ml) from 500g of cottonseed and heated to 90°C and coagulated using calcium chloride at three different concentrations: 0.111 g, 0.222 g, and 0.333 g. After coagulation, the protein was separated and weighed. The respective protein yields obtained were 11.2 g, 14.3 g, and 14.1 g.



Fig:3.7a



Fig:3.7b



Fig:3.7c



Fig:3.7d

Table.2 Experimental Trials for Cottonseed Milk Protein Coagulation (using Bleaching Agent)

S.No.	Parameter	Trial 7	Trial 8
1	Cottonseed used for extraction	100g	200g
2	Amount of bleaching agent	0.015g	0.025g
3	Cottonseed milk used	100ml	200 ml
4	Temperature	90°C	90°C
5	Amount of coagulant	0.222g	40 ml
6	Yield	16.2 g	22.5

Trial 7

Cottonseed was treated with potassium metabisulphite (0.015g) for bleaching (first stage) by soaking for 10 minutes, followed by washing. Cottonseed milk (100ml) was then extracted and heated to 90°C. Calcium chloride (0.222 g) was added as the coagulant, resulting in a protein yield of 16.2 g.



Fig:3.8



Fig:3.8b

Trial 8

Cottonseed was bleached (second stage) using potassium metabisulphite (0.025g), then washed and used for milk extraction. Cottonseed milk (200 ml) from 200g of cottonseed and heated to 90°C, and vinegar (40 ml) was added as the coagulant. The protein yield obtained was 22.5 g. The final product was sent for laboratory analysis of free gossypol, protein, and ash content.



Fig:3.9a



Fig:3.9b

Analysis of Cottonseed Protein

The developed cottonseed protein product was analyzed for protein content, ash content, pH, and gossypol content to evaluate its nutritional quality and safety characteristics. The protein content of the developed product was found to be 21.3%, indicating that the product is a rich source of plant-based protein. This value was found to be higher than the protein content reported for dairy paneer ($17.61 \pm 0.21\%$) and also higher than the typical protein range reported for soy paneer 8–15%, depending on processing conditions as reported by (Hemant Gawande et al., 2023). The higher protein content obtained in the present study may be attributed to effective protein extraction, optimized heat treatment, and efficient coagulation conditions used during processing. These findings indicate that the developed cottonseed protein product has the potential to serve as a protein-rich alternative to both conventional dairy paneer and other plant-based substitutes.

The ash content of the product was found to be 6.99%, indicating the presence of mineral constituents in the developed product. Ash content represents the total mineral matter present after complete combustion of organic components and may include minerals such as calcium, phosphorus, potassium, magnesium, and other trace elements naturally present in cottonseed or introduced during processing. The presence of these minerals contributes to the nutritional value of the final product.

The pH of the cottonseed protein product was found to range between 6.0 and 6.5, indicating that the product is moderately less acidic in nature. This pH range was observed to be higher than that of dairy paneer, which generally falls between 5.3 and 5.8, while remaining comparable to soy paneer, which typically ranges from 6.0 to 7.0. The relatively neutral pH of the developed product may influence important quality attributes such as texture, coagulation behavior, microbial stability, and storage characteristics. The pH value also suggests that the product possesses processing characteristics similar to other plant-based protein products.

Gossypol analysis was carried out to assess the safety of the developed cottonseed protein product. Gossypol is a naturally occurring polyphenolic compound present in cottonseed and exists in two forms: free gossypol, which is toxic, and bound gossypol, which is comparatively less harmful. In the present study, free gossypol values ranging from 1.32–1.41% and bound gossypol of 1.88% were observed. The presence of bound gossypol indicates that a portion of the gossypol may have interacted with proteins during processing and converted into a less toxic form. However, the persistence of free gossypol indicates incomplete detoxification and raises concerns regarding product safety.

The total gossypol content was found to be 1.23% in cottonseed milk and 1.36% in the extracted protein, showing that considerable amounts of gossypol remained even after bleaching, heat treatment, and coagulation processes. According to the Food Safety and Standards Authority of India (FSSAI), the permissible limit of free gossypol in edible cottonseed products is 450 ppm (0.045%). The values observed in the present study were significantly higher than the recommended safety limits, indicating that the developed product is not suitable for direct consumption in its current form. Therefore, further

detoxification and advanced processing techniques are required to effectively reduce gossypol levels and improve the safety of cottonseed-derived protein products for human consumption.

V.CONCLUSION

The present study was carried out with the objective of developing a protein-rich plant-based product from cottonseed through the preparation of cottonseed milk and subsequent protein coagulation. In recent years, there has been a growing demand for vegan and plant-based protein products due to increasing awareness about health, sustainability, lactose intolerance, and the need for alternative protein sources. In this context, cottonseed was explored as a potential underutilized raw material for the development of a value-added protein product.

During processing, both unbleached and bleached cottonseed samples were evaluated. Initially, the unbleached cottonseed milk showed a darker colour, which could affect the appearance and acceptability of the product. Therefore, bleaching treatment using potassium metabisulphite was carried out to improve the colour and overall quality of the cottonseed milk. The first-stage bleaching resulted in only a mild colour improvement, while the second-stage bleaching showed better lightness and improved visual appearance. Further processing involved heat treatment at 80–90°C and coagulation using different coagulants such as lemon juice, vinegar, and calcium chloride to obtain a tofu-like protein product.

The developed product was further subjected to laboratory analysis to evaluate its nutritional quality and safety. The protein content of the final product was found to be 21.3%, indicating that the developed product is a good source of plant-based protein. The ash content was found to be 6.99%, showing the presence of mineral content in the sample. Gossypol analysis revealed that the gossypol content remained high even after processing. In the initial analysis, gossypol levels were found to be 1.23% in cottonseed milk and 1.36% in cottonseed coagulates. Further analysis showed free gossypol values of 1.32% and 1.41%, while bound gossypol was found to be 1.88%. These results indicate that the treatments applied during processing, including bleaching, heat treatment, and coagulation, were not sufficient to significantly reduce gossypol levels.

In conclusion, the study successfully demonstrated the feasibility of producing a cottonseed-based protein product with good protein content and improved quality characteristics. However, since gossypol is a toxic anti-nutritional compound, the product is not suitable for direct consumption in its present form and requires further detoxification and advanced processing techniques to reduce gossypol to safe levels before it can be recommended for human consumption.

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