Journal of Applied and Natural Science
16(4), 1656 - 1661 (2024)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Extraction, characterization, and functional properties of ultra-low gossypol protein from cottonseed meal

Preeti Goyal*
Department of Biochemistry, Punjab Agricultural University, Ludhiana (Punjab), India
Dattatreya M. Kadam
ICAR-Central Institute for Research on Cotton Technology, Mumbai (Maharashtra), India
Akansha Kasara
ICAR-Central Institute for Research on Cotton Technology, Mumbai (Maharashtra), India
Sayali Parab
ICAR-Central Institute for Research on Cotton Technology, Mumbai (Maharashtra), India

*Corresponding author. E-mail: preetigoyal@pau.edu

Article Info
https://doi.org/10.31018/jans.v16i4.5918
Received: July 4, 2024
Revised: November 23, 2024
Accepted: November 28, 2024

How to Cite
Goyal, P. *et al.* (2024). Extraction, characterization, and functional properties of ultra-low gossypol protein from cottonseed meal. *Journal of Applied and Natural Science*, 16(4), 1656 - 1661. https://doi.org/10.31018/jans.v16i4.5918

Abstract
Cottonseed protein (CSP) is unsuitable for human consumption due to its gossypol toxicity. The present study aimed to extract protein from cottonseed meal with ultra-low gossypol content and evaluate its functional properties for food products. The study developed a protein extraction process with gossypol removal treatment to unlock its potential to generate cottonseed meal protein isolate with ultra-low gossypol content.Key factors, including the pH of the extraction solvent time washing, and drying of protein pellets, were optimized to improve protein yield and reduce gossypol content. Extraction was performed using 0.15 M NaCl, 0.27% Na_2SO_3, and 0.1 M KOH at pH-12 for 2 hours at ambient temperature. The protein isolate yielded a recovery rate of 73.9%, with free and total gossypol concentrations of 348 ppm and 4170 ppm, respectively. The isolate demonstrated excellent functional properties, including a water holding capacity of 3.795 ± 0.987 ml/g, oil absorbing capacity of 2.069 ± 0.103 ml/g, foaming capacity of 500%, and stability of 88%.The present work lies in the successful extraction of a gossypol-free protein isolate with promising functional properties, making it suitable for preparing various value-added food products such as biscuits and cakes.

Keywords: Cottonseed meal protein isolate (CSMPI), Extraction, Functional properties, Gossypol content, Ultra-low gossypol protein

INTRODUCTION

Cotton has been cultivated for its fibre traditionally for ages. India produces 22% of world cotton production. Besides fibre, the cotton plant produces seeds. Cottonseeds are a source of oil and protein. The protein content of cottonseed (23%), cottonseed meal (CSM) (30-50%), and Cottonseed meal protein isolate CSMPI (80%) is very high, which makes it an excellent source of protein to meet the global protein demand for livestock and human consumption (Rathore *et al*., 2017, Tan *et al*., 2022). However, the major constraint in its wider utilization for food or even as feed is the presence of gossypol which is produced throughout the cotton plant via pigment glands. Gossypol is constitutively present, and its production is amplified as a natural defence compound during an attack by microorganisms, insects, and pests (Bezemer *et al*., 2004). It is a polyphenolic compound that occurs in free and bound (lysine and arginine) forms (Ma *et al*., 2018). Gossypol, consumed beyond FDA recommendation (450 ppm and 12000 ppm in free and bound form, respectively) is found to have a detrimental effect on the health of dairy cows (Zhang *et al*., 2007) and broiler chicks (Henry *et al*., 2001), Dogs (Uzal *et al*., 2005) and humans (Gadelha *et al*., 2014). Different practices for gossypol removal as microbial fermentation (Zhang *et al*., 2018) and solvent-based cottonseed protein extraction (Pelitire *et al*., 2014, Singh *et al*., 2019 and Kumar *et al*., 2021) are being used.

Cottonseed meal is a rich source of protein and fibre but due to the presence of gossypol, it is used in non-feed or non-food industries. Solvent-based cottonseed protein extraction with low gossypol has been an im-

portant area of research for a long time. Suppose protein extracted from cottonseed meals had a gossypol concentration lower than the Food and Drug Administration (FDA) recommendation. In that case, it can be used by food industries, which is certainly a new source of edible protein for forming value-added products. Osti and Pandey (2006) used cottonseed meal as a protein source in the diets of ruminants and observed that CSM protein digestibility and degradability are similar to soybean meal, peanut meal, and canola meal. Food products like snacks, baked goods, etc. have been developed using CSM protein. The major factor that limits the use of CSM protein in food products is the presence of gossypol in high concentrations. We have improved the alkali-based CSM protein isolation approach, followed by protein precipitation with citric acid prescribed by Kumar *et al*. (2021) to decrease the gossypol level.

Cottonseed meals contain globulins (50%), albumin (28%), Glutelin (16%), and prolamin (6%), as reported by Singh and Kaur (2019). Globulins are soluble in the salt solution, albumins in water, glutelin in dilute alkali, and prolamin in 60-70% ethanol (deMan *et al*., 2018). Therefore, the extraction solvent contains NaCl, Na2SO3, and alkali KOH salts in distilled water to obtain most of the proteins. The CSM protein isolate was further characterized for total protein, bound, and free gossypol concentration. An analysis of their functional properties is very important for the preparation of value-added products from CSM protein isolate. Besides nutritional properties, functional properties explain how our added nutrient will behave during preparation and cooking and how the finished product looks, feels, and tastes. Functional properties of the cottonseed protein isolate, like emulsifying and stability, foaming and stability, Oil absorbing and water holding capacity, were studied to develop value-added products. The present study aimed to use protein isolated from cottonseed meal as a food source for human beings that is otherwise used as feed for livestock or non-food industries. It would be beneficial for the growth of society and the economy to have another source of protein.

MATERIALS AND METHODS

Materials

The defatted cottonseed meal was provided by Pitchampalayam, Tiruppur, Tamil Nadu, India, and was used to produce cottonseed protein isolate after grinding with a pestle and mortar and sieve through 60 mesh to remove impurities and ensure uniformity. All the chemicals and reagents were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MA, USA), Himedia Laboratories & Genex Life Sciences Pvt. Ltd., Mumbai, India, and Merck Specialities Pvt. Ltd., Germany.

For the extraction of cottonseed protein, the method of Kumar *et al*. (2021) was followed with certain modifications. Alkali-based extraction solvent was prepared with 0.15M NaCl, 0.27% Na_2SO_3, and 0.1M KOH. CSM at a flour-to-solvent ratio of 1:32 was extracted for 2, 4, and 24 hours with continuous stirring in the orbital shaker at ambient temperature. To study the effect of pH and temperature, extraction was performed by adjusting the pH of the extraction solvent around 10.0 and 11.0 as well as on pH 12 and temperature 4, 10, and 25 □. While the pH of the extraction solvent was found to be 12. The effect of pH as well as time of extraction on protein recovery and gossypol concentration was observed. Then, CSM plus extraction solvent mixture was centrifuged at 15000 rpm for 15 minutes. The supernatant was collected in a beaker and the pellets were discarded.

Protein was precipitated based on solubility. Citric acid was used for protein precipitation. Solid citric acid was added slowly to the supernatant with constant shaking on a magnetic stirrer at ambient temperature to bring the pH to 4.5. The stirring was continued for another few minutes, and the solution was kept in a refrigerator overnight to precipitate the proteins completely. After that, the solution was centrifuged at 15000 rpm for 15 min. The protein pellets were collected and the supernatant was discarded.

To study the effect of pH, temperature, and time of extraction solvent on protein yield, the extraction of protein was carried out with pH 10.0, 11.0, and 12.0 at temperatures of 4, 10, and 25 □ for 2, 4, and 24 hours and precipitation of protein was done respectively.

Characterization of cotton seed meal protein Isolate (CSMPI)

Crude protein contents

Crude protein content was calculated by multiplying % nitrogen by 6.25. Nitrogen in flour was estimated by Micro-Kjeldahl's method (Association of Official Analytical Chemists, AOAC, 2000)

Free Gossypol: Free gossypol content of CSM and CSPI was determined according to AOCS official method number Ba 7–58 (2017).

Total Gossypol: Free gossypol content of CSM and CSPI was calculated according to AOCS official method number Ba 8–78 (2017).

Functional properties

Water and oil holding capacity was determined by the procedure explained by Ma *et al*., 2018. Foaming capacity and foam stability were determined using the modified method of Timilsena *et al*. (2016). Emulsifying capacity and stability was calculated by the method of Ma *et al*., 2018.

Water and oil holding capacity, emulsifying capacity and stability were determined by the procedure ex-

plained by Ma *et al*. (2018). Foaming capacity and foam stability were determined using the modified method of Timilsena *et al*. (2016).

RESULTS AND DISCUSSION

Gossypol-free cottonseed protein was successfully isolated and characterized, with content lower than FDA recommendations (Ma *et al*., 2018). The functional properties of cottonseed meal protein isolate (CSMPI) were analyzed to evaluate its suitability as a potential food source for human consumption.

Effect of extraction solvent, pH, temperature, and time on protein extraction, precipitation, and recovery yield

An alkaline medium was used for the extraction of proteins from cottonseed meals. The extraction solvent contained salts sodium chloride (NaCl), sodium sulfite (Na_2SO_3), and alkali KOH. Sodium chloride breaks the ionic bond between proteins and phenolic compounds and helps to extract protein. Sodium sulfite breaks down disulfide linkage and reduces chemical crosslinking (Xu and Diosady, 2002).Potassium hydroxide was added to dissolve the cellulose and lignin, which increases protein solubility. The effect of extraction solvent, pH, temperature, and time on protein extraction, precipitation, and recovery yield is presented in Fig. 1. The pH of the extraction medium was 12. There was no need to adjust the pH of the extraction solvent to 10.0 – 11.0 due to a decrease in the protein recovery at pH 10.0-11.0 . The extraction was performed at 4, 10, and 25□. The recovery per cent of proteins was nearly equal at ambient and low temperatures. Maximum yield of protein, i.e., 73.9% was observed when extracted for 24 hr. (Fig.1). Protein yield was consistent with Kumar *et al*. (2021) and He *et al*. (2013), while lower protein recovery was observed in rice bran and sesame (Yilmaz and Dilek, 2016, Phongthai *et al*., 2016).

Characterization of cotton seed meal protein Isolate CSMPI

Higher content of crude protein was observed in both CSM and CSPI by Kumar *et al*. (2021), with a recovery of 93.6% from CSM and an experimental yield of 88.5% for CSPI, which had a free gossypol concentration of 29.0 ppm. In contrast, lower crude protein content (40-60%) was observed in soy protein (He *et al*., 2013).

Total gossypol and free gossypol content (%) of CSMPI was 4170 ± 25 and 348 ± 5 ppm, respectively (Fig. 2). In the CSM the total gossypol and free gossypol content was 20900 and 900 ppm, respectively. The extraction and precipitation procedure indicated a reduction in the gossypol level in the protein isolate. The present study used citric acid instead of ammonium sulfate to use the precipitated protein as a food source. Citric acid has also been found to hydrolyze the Schiff base linkage between the gossypol and amino group of lysine (Pelitire *et al*., 2014). Earlier free and total gossypol content, i.e., 29 ± 1.3 ppm and 270 ± 21.0 ppm, is reported in CSMPI (Kumar *et al*., 2021). Gossypol, a polyphenolic terpenoid found in cotton plants, belongs to the genus Gossypium and the family Malvaceae (Aharoni *et al*., 2005). It served as a defence agent for the plant but, when fed to animals, was found to be toxic for them. The permissible limit of free and total gossypol is 0.045%/450 ppm and 1.2%/12000 ppm (Ma *et al*., 2018; Zhang *et al*., 200).

Functional properties

Water holding capacity (WHC) is an important parameter related to food texture. CSPMI has a 3.795 ± 0.987 g/g protein water holding capacity, while a value of 6.29 g/g protein was reported in soy protein isolate (Zhang and Zhao, 2013). WHC is vital in food formulation as high WHC may dehydrate other ingredients in the food system and low WHC affects the food product storage (Haque, 2016). The oil binding capacity of protein is its ability to retain lipids in food formulation and it affects the formation of emulsions, flavor absorption, and dough preparation. The oil binding capacity of CSMPI was found to be 2.069 ± 0.103 g/g of protein. Zhang and Zhao (2013) reported a value of 4.00 and 2.69 ml/g in soy protein isolate and modified soy protein isolate, respectively. Variations in values may be due to different plant sources, temperature, extraction, and precipitation conditions (Zayas, 1997).

In the present study, CSMPI was evaluated for use in foods such as beverages, ice cream, dressings, whipped toppings, and biscuits, where emulsifying and foaming properties of the protein are essential. The Emulsifying Activity Index (EAI) and Emulsifying Stability Index (ESI) of CSMPI were found to be 23.693 ± 0.512 m²/g and 74.667 ± 0.812 minutes, respectively, which are comparable to those of soy protein isolate (Zhang and Zhao, 2013). EAI is the amount of oil emulsified by one gram of protein and ESI is the amount of oil separated from an emulsion during a certain period at ambient temperature (Aryee *et al*., 2018). In the pre-

Table 1. Functional properties of cottonseed meal protein isolate

Parameter	Quantity*
Water holding capacity (g/g	3.795 ± 0.987
Oil absorbing capacity (g/g	2.069 ± 0.103
Emulsifying activity index (m^2/g)	23.693 ± 0.512
Emulsifying stability (min)	74.667 ± 0.812
Foaming capacity (%)	500 ± 2.5
Foam stability (%)	88 ± 1.1

*Results are mean ± standard deviations of duplicate analysis

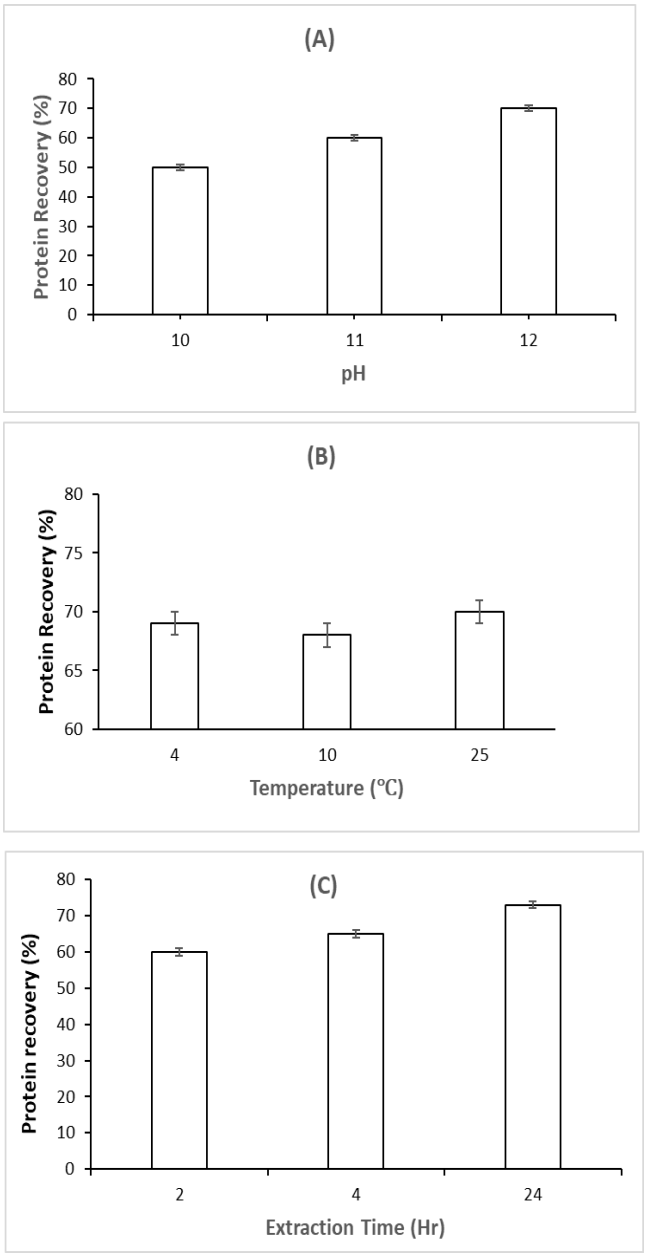

Fig. 1. *Effect of pH (A), Temperature (B) and Extraction time (C) on protein yield*

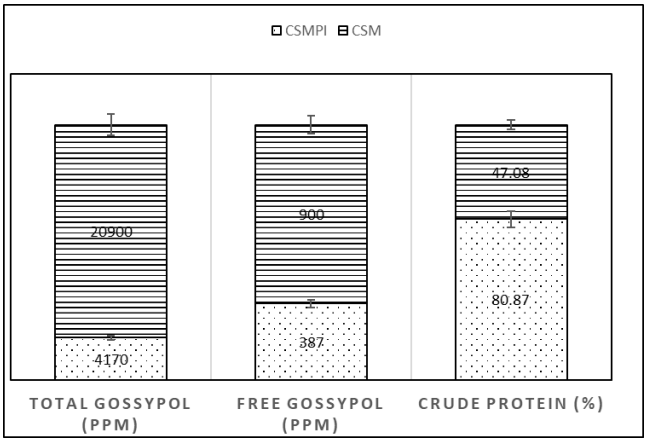

Fig. 2. *Gossypol and crude protein content of Cottonseed meal protein*

sent study, the foaming properties of CSMPI were evaluated, revealing a remarkably high foaming capacity of 500%. Foam stability, measured as the percentage of volume retained after 30 minutes, was 88%. These results indicate that CSMPI significantly enhances both the smoothness and palatability of aerated food products, making it highly suitable for applications requiring superior foam formation and stability.

Earlier very low value of foaming capacity (80%) and comparable foam stability (80-90%) was reported in cottonseed meal protein isolate (Ma *et al*., 2018). The protein solution was found to be a potent foaming agent isolated from mungbean (Du *et al*., 2018). In the present study, CSMPI exhibited a water holding capacity of 3.795 g/g protein, oil absorbing capacity of 2.069 g/g protein, emulsifying activity index of 23.693 m²/g, and emulsifying stability of 74.667 minutes. These functional properties indicate that CSMPI could be a valuable ingredient in food formulations, particularly in products requiring enhanced moisture retention and fat absorption. Additionally, CSMPI demonstrated a remarkable foaming capacity of 500% and foam stability of 88%, significantly outperforming previously reported plant protein isolates.

When compared to recent studies, the results of this study align with the growing body of research on plant-based protein functionality. Tan *et al*. (2022) reported that their CSMPI exhibited superior oil absorption capacity and water solubility compared to pea protein isolate, which is consistent with our findings of high water holding and oil absorbing capacities. Moreover, their degossypolization process effectively reduced gossypol levels to well below the US FDA limit, supporting the safety and potential of CSMPI for human consumption. Similarly, Kumar *et al*. (2021) emphasized that chemical and biological methods of degossypolization could enhance the usability of cottonseed protein for food and feed applications. Our findings build on this by demonstrating that CSMPI retains strong functional properties after degossypolization.

Cheng *et al*. (2020) noted the increasing interest in cottonseed protein due to its availability and versatility in food and non-food applications. The present study contributes to this growing interest by demonstrating that CSMPI has industrial potential and exhibits enhanced functionality compared to other plant proteins. This study's high foaming capacity and stability suggest that CSMPI could be used in aerated food products, such as whipped toppings and dairy substitutes, where these properties are critical.

In addition to its functional advantages, the amino acid analysis performed by Tan *et al*. (2022) showed that CSMPI is rich in branched-chain amino acids (BCAAs), making it a nutritionally valuable protein source. This aligns with the increasing demand for plant-based pro-

teins with balanced amino acid profiles. Furthermore, their allergenic prediction studies and digestibility analysis suggest a low likelihood of CSMPI allergenicity, reinforcing its suitability for human consumption.

The present study demonstrates that CSMPI, with its excellent functional properties and low gossypol content, can be a promising alternative protein for human food applications. Its high water-holding capacity, oil absorption, and foaming properties make it suitable for use in baked goods, dairy alternatives, and other food products requiring enhanced texture and stability. This study adds to the body of literature supporting the potential of cottonseed protein as a sustainable, functional, and nutritious ingredient for the food industry.

Conclusion

In the present study, the extraction and precipitation of cottonseed protein isolate (CSMPI) using alkali and acid, respectively, were optimized, with the best conditions determined as a flour-to-sample ratio of 1:32, pH of 12, and a temperature of 25°C, yielding 73% protein. The characterization of the isolate revealed ultralow gossypol content, making it safe for human consumption. Additionally, the functional properties, including water-holding capacity, emulsifying capacity, emulsifying stability, and foaming properties, were found to be excellent, demonstrating CSMPI's potential as a valuable protein source in various food products. These results suggest that CSMPI can be effectively utilized as an ingredient in value-added products. However, further experiments are required to fully explore its applications and evaluate its functional properties in various food formulations.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support by the Department of Science and Technology, Ministry of Science and Technology, and technical support and resources of ICAR-Central Institute for Research on Cotton Technology, Mumbai.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Aharoni, A., Jongsma, M. A., & Bouwmeester, H. J. (2005). Volatile science? Metabolic engineering of terpenoids in plants. *Trends in Plant Science*, 10(12), 594-602. https://doi.org/10.1016/j.tplants.2005.10.005
2. AOAC (2000). Method 992.23, In *Official Methods of Analysis* 17th Ed., AOAC International, Gaithersburg, MD.
3. AOCS (2017). Method Ba 7-58, In *Official Methods of Journal of the American Oil and Chemist's Society*, 8th Ed., Champaign, IL 61822.
4. AOCS (2017) Method Ba 8-78, In *Official Methods of Journal of the American Oil and Chemist's Society*, , 8th Ed., Champaign, IL 61822.
5. Aryee, A. N. A., Agyei, D., & Udenigwe, C. C. (2018). Impact of processing on the chemistry and functionality of food proteins. In *Proteins in food processing*, Woodhead Publishing, pp. 27-45. https://doi.org/10.1016/B978-0-08-100722-8.00003-6.
6. Bezemer, T. M., Wagenaar, R., Van Dam, N. M., Van Der Putten, W. H., & Wäckers, F. L. (2004). Above-and below-ground terpenoid aldehyde induction in cotton, Gossypium herbaceum, following root and leaf injury. *Journal of Chemical Ecology*, 30(1), 53-67. https://doi.org/10.1023/B:JOEC.0000013182.50662.2a.
7. Cheng, H. N., Zhongqi, He., Ford, C., Wycko, W. & Wo, Q. (2020). A Review of Cottonseed Protein Chemistry and Non-Food Applications. *Sustainable Chemistry*, (1), 256–274 doi:10.3390/suschem1030017
8. deMan, J. M., Finley, J. W., Hurst, W.J., & Lee C. Y. (2018). In *Principles of food chemistry*, 4th edition, Springer, Switzerland)
9. Du, M., Xie, J., Gong, B., Xu, X., Tang, W., Li, X., Li, Chang., & Xie, M. (2018). Extraction, physicochemical characteristics and functional properties of Mung bean protein. *Food Hydrocolloid*, (76), 131-140. https://doi.org/10.1016/j.foodhyd.2017.01.003.
10. Gadelha, I. C. N., Fonseca, N. B. S., Oloris, S. C. S. Melo, M. M., & Soto-Blanco, B. (2014). Gossypol Toxicity from Cottonseed Products. *The Scientific World Journal*, (2014), 231635, 11. https://doi.org/10.1155/2014/231635.
11.
12. Haque, M. A., Timilsena, Y. P., & Adhikari, B. (2016). Food Proteins, Structure, and Function, In *Reference Module in Food Science*, Elsevier, ISBN 9780081005965, https://doi.org/10.1016/B978-0-08-100596-5.03057-2.
13. He, Z., Cao, H., Cheng, H. N., Zou, H., & Hunt, J. F. (2013). Effects of vigorous blending on yield and quality of protein isolates extracted from cottonseed and soy flours. *Modern Appllied Science*, 7(10), 79–88. http://dx.doi.org/10.5539/mas.v7n10p79
14. Henry, M. H., Pesti, G. M., & Brown, T. P. (2001). Pathology and histopathology of gossypol toxicity in broiler chicks. *Avian Diseases*, 45(3), 598–604. https://doi.org/10.2307/1592900.
15. Kumar, M., Tomar, M., Punia, S., Grasso, S., Arrutia, F., Choudhary, J., Singh, S., Verma, P., Mahapatra, A., Patil, S., Radhika, Dhumal, S., Potkule, J., Saxena, S. & Amarowica, R. (2021). Cottonseed: A sustainable contributor to global protein requirements, *Trends in Food Science and Technology*, (111), 100-113. https://doi.org/10.1016/j.tifs.2021.02.058
16. Kumar, M., Potkule, J., Patil, S., Saxena, S., Patil, P. G., Mageshwaran, V., Punia, S., Varghese, E., Mahapatra, A., Ashtaputre, N., & Kennedy, J. F. (2021). Extraction of ultra-low gossypol protein from cottonseed: Characterization based on antioxidant activity, structural morphology and functional group analysis. *LWT*, (140), 110692. https://doi.org/10.1016/j.lwt.2020.110692
17. Ma, M., Ren, Y., Xie, W., Zhou, D., Tang, S., Kuang, M., Wang. Y., & Du, S. K. (2018). Physicochemical and func-

tional properties of protein isolate obtained from cottonseed meal. *Food Chemistry*, (240), 856–862. https://doi.org/10.1016/j.foodchem.2017.08.030

18. Osti, N. P., & Pandey, S. B. (2006). Use of whole cotton seed and cotton seed meal as a protein source in the diet of ruminant animals: Prevailing situation and opportunity. In: *proceedings of the 6th National Workshop on Livestock and Fisheries Research. Lalitpur: National Animal Science Research Institute (NASRI)*, Nepal, p. 111–119.
19. Pelitire, S. M., Dowd, M. K., & Cheng, H. N. (2014). Acidic solvent extraction of gossypol from cottonseed meal. *Animal Feed Science and Technology*, (195), 120-128. https://doi.org/10.1016/j.anifeedsci.2014.06.005
20. Phongthai, S., Lim, S. T., & Rawdkuen, S. (2016). Optimization of microwave-assisted extraction of rice bran protein and its hydrolysates properties. *Journal of Cereal Science*, (70), 146–154. https://doi.org/10.1016/j.jcs.2016.06.001
21. Rathore, K. S., Wedegaertner, T. C., & Hake, K. (2017). Ultra-low gossypol cottonseed (ULGCS) as a feed for non-ruminants to enhance human nutrition security. In *Broadening Horizons. Feedipedia-Animal Feed Resources Information System-INRA CIRAD AFZ and FAO.* https://www.feedipedia.org/content/ultra-low-gossypol-cottonseed-ulgcs-feed-non-ruminants-enhance-human-nutrition-security.
22. Singh, A., & Kaur, A. (2019). Comparative studies on seed protein characteristics in eight lines of two Gossypium species. *Journal of Cotton Research*, 2(1), 1-12. https://doi.org/10.1186/s42397-019-0024-3
23. Singh, S., Sharma, S. K., & Kansal, S. K. (2019). Batch extraction of gossypol from cottonseed meal using mixed solvent system and its kinetic modeling. *Chemical Engineering Communications*, 206(12), 1608-1617. https://doi.org/10.1080/00986445.2018.1558214
24. Tan, C. F., Kwan, S. H., Lee, C. S., Soh, Y. N. A., Ho, Y. S., & Bi, X. (2022). Cottonseed Meal Protein Isolate as a New Source of Alternative Proteins: A Proteomics Perspective. *International Journal of Molecular Science*, 23 (17), 10105. https://doi.org/ 10.3390/ijms231710105.
25. Timilsena, Y. P., Adhikari, R., Barrow, C. J., & Adhikari, B. (2016). Physicochemical and functional properties of protein isolate produced from Australian chia seeds. *Food Chemistry*, (212), 648-656. https://doi.org/10.1016/j.foodchem.2016.06.017
26. Uzal, F. A., Puschner, B., Tahara, J. M., & Nordhausen, R. W. (2005). Gossypol toxicosis in a dog consequent to ingestion of cottonseed bedding. *Journal of Veterinary Diagnostic Investigation*, 17(6), 626-629. https://doi.org/10.1177/104063870501700622
27. Xu, L., Lui, F., Luo, H., & Diosady, L. L. (2003). Production of protein isolates from yellow mustard meals by membrane processes. *Food Research International*, 36(8), 849-856. https://doi.org/10.1016/S0963-9969(03)00082-6
28. Yilmaz, E., & Emir, D. D. (2016). Extraction and functional properties of proteins from pre-roasted and enzyme treated poppyseed (*Papaver somniferum L.*) press cakes. *Journal of Oleo Science*, 1–11.
29. Zayas, J.F. (1997). Oil and Fat Binding Properties of Proteins. In *Functionality of Proteins in Food*. Springer, Berlin, Heidelberg., https://doi.org/10.1007/978-3-642-59116-7_5.
30. Zhang, W. J., Xu, Z. R., Pan, X. L., Yan, X. H., & Wang, Y. B. (2007). Advances in gossypol toxicity and processing effects of whole cottonseed in dairy cows feeding. *Livestock Science*, 111(1-2), 1-9. https://doi.org/10.1016/j.livsci.2007.03.006
31. Zhang, Y. N., & Zhao, X. H. (2013). Study on the functional properties of soybean protein isolate cross-linked with gelatin by microbial transglutaminase, *International Journal of Food Properties*, 16(6), 1257-1270. DOI: 10.1080/10942912.2011.583705.
32. Zhang, Y., Zhang, Z., Dai, L., Liu, Y., Cheng, M., & Chen, L. (2018). Isolation and characterization of a novel gossypol-degrading bacteria Bacillus subtilis strain Rumen Bacillus Subtilis. *Asian-Australasian Journal of Animal Sciences*, 31(1), 63. https://doi.org/10.5713%2Fajas.17.0018