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A comprehensive review on detoxification of cottonseed

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Abstract
Over 10 million metric tons of protein are produced worldwide from cottonseed, making it a sustainable source of plant protein. But it also contain gossypol, which is a toxic compound that can adversely affect the quality of the protein isolate. A cottonseed that contains less than 0.45% gossypol is said to be quite suitable for human and animal consumption, as it contains a high amount of protein, edible oil, and energy. Degossypolyzation processes still heavily rely on solvent extraction, although gamma irradiation and the use of microorganisms are gaining popularity. Degossypolization and better cottonseed protein (CSP) use for human nutrition may both benefit from the integration of chemical and biological approaches. In this review, techniques are described that can enhance the quality of cottonseed meal for human nutrition by lowering the gossypol content to levels that are within acceptable ranges (450 ppm). The findings of this study suggest that detoxification is a crucial step in improving the quality of cottonseed protein isolate, and the use of the alkali treatment method can be a promising approach for this purpose. Additionally, it also provide valuable insights into the potential applications of protein of cottonseed isolate in food industries and highlight the importance of appropriate detoxification methods to enhance its functionality. This research can contribute to the development of a sustainable and cost-effective source of protein for various food applications.

Keywords: Cottonseed, gossypol, detoxification, malnutrition, protein

Introduction
Africa and Asia are lacking behind in terms of malnutrition. 43% of malnourished children of age under five years were found in India which means 61 deaths per thousand. While other countries are also having the same data as Bangladesh has 41%, Afghanistan has 33%, Pakistan has 31%, Nepal has 29%, Myanmar has 23%, Sri Lanka has 22%, Thailand has 7% and China has 3%. Aa stated by UNICEF (United Nations Childrens fund), Children under the age of five are also malnourished to a range of high (30 - 39%) to very high (>40%), according to the World Health Organization. Because this issue has been a concern for the people of these nations since ancient times, it has persisted into the current generation and cannot be solved at this time. Agro-food waste and byproducts of the food industry are increasingly being reprocessed to address the problem of expanding populations and resource depletion (Gemechu, 2020) [15]. As a result, cottonseed, a by-product of the oil manufacturing, is probably going to be employed as an unconventional and affordable source of protein and oil. Cottonseed meal (CSM) is rich source of protein (40-45%) with a healthy balance of the essential amino acids and remains grossly underutilized. Worldwide around Every year, 10–11 million metric tons of cottonseed protein are produced. The protein fraction found in cottonseed includes proteins that are alkali soluble (glutelins 9.2-28%), water soluble (albumins 20.8-32.2%), and salt soluble (globulins 33-63%). A game-changer in reducing malnurition will be the successful and effective use of cottonseed as a protein source in the majority of the affected countries (Kumar *et al*., 2021) [22]. Based on these considerations, it can be used as a potential protein source to fulfill Industrial demand for a cheap raw material to replace more expensive sources of protein. Nevertheless, it contains gossypol, which can be harmful if consumed in large amounts. In every part of cotton plants a toxic polyphenolic compound is present, but the highest concentration of Gossypol is present in cotonseeds that is upto dry on weight basis 2.4%. Due to the toxin's presence in the diet, numerous negative impacts were found, including detrimental effects on animal growth, development, and reproductive livestock health. A safe consumption level of gossypol can be achieved in CSM if the gossypol content is reduced. Given the circumstances, detoxification has developed into a crucial step in the processing of seed kernels (Zhang *et al*., 2006) [33]. This step allows the level of these components to be lowered to safe levels while also removing the bitterness and potential toxicity.

There are two types of procedures that are typically used to detoxify apricot kernels: traditional methods and cutting-edge approaches. Gossypol can be removed from CSM chemically, allowing it to be used as a feed for non-ruminants and as a nutritional supplement for humans. Additionally, solvent-based procedures resulted in a decrease in gossypol levels in CSM, making it suitable for use in fisheries, poultry farms, and pig farms (Rathore *et al*., 2020) [29]. By acidifying ethanol and acetone with phosphoric acid, 90–95% of the total gossypol in the CSM could be reduced successfully (Pelitire *et al*., 2014) [26]. By contrast, a biological approach involving different microbial strains and enzymes can be used to reduce gossypol concentrations in CSM in an environmentally friendly manner (Kumar *et al*., 2021) [22]. In this regard, it is necessary to conduct some research concerning the impact of treatment methods on protein quality prior to re-evaluating these treatments for kernel detoxification since it is a highly nutritious source of protein that contains large quantities of essential amino acids. To our knowledge, there isn't any information available in this situation. Therefore, the main objective of this study to determine the potential detoxification techniques for cottonseed meal and whether detoxified meal can be reused for the production of affordable nutritional products.

Detoxification methods

Physical Methods

Depending on the variety of cotton, the area and climatic conditions in which the cotton crop is grown, cottonseed has a dry weight basis concentration of gossypol ranging from 0.002 to 6.64%. (Gadelha *et al*., 2014) [13]. The size of the glands of resin on the cotton plant ranges from 50 to 400 m. These glands are found various parts of plants, and they produce gossypol (Gardner *et al*., 1976) [14]. The most abundant source of Gossypol is cotton seed kernels, where it is present in amounts of 0.8 to 2% by dry weight. Natural monomer sesquiterpenoids are produced by cotton plants and have been classified as dimeric sesquiterpenoids since they are produced by dimerizing hemi-gossypol molecules (Cai *et al*., 2010) [5]. In the past, gossypol was extracted or removed from cottonseed using physical methods such as air classification (Decossas *et al*., 1982) [8], liquid cyclone (Smith, 1971) [32], and gland flotation (Boatner *et al*., 1949) [4]. Physical characteristics, such as density differences, and natural forces, such as gravity, were used in these techniques These two methods enabled the isolation of glands containing gossypol from the CSM (Singh *et al*., 2015) [31]. The gland flotation method uses solvents whose density is lower than that of pigment glands in order to separate gossypol-contained glands and kernel tissues. The slurry was then disappeared from the glands, and the top layer was collected while the sludge was left to stand. Gossypol removal/extraction from cottonseed was once a common practice. The cottonseeds were mixed with a suspension of low-moisture solvents during the liquid cyclone process, and after passing through a colloidal mill, the gossypol-containing glands were evenly disseminated without being shattered. Using gravity, sedimentation, flotation, or any combination thereof, these resin glands were isolated from CSM. According to Gardner *et al*. (1976) [14], the liquid cyclone technique may generate edible CSM with more than 65% protein and fewer than 400 ppm free gossypol. The liquid cyclone technique began commercial production in Texas following USFDA certification. Despite its advantages, this procedure had little commercial viability and was thus no longer in use for a short while. The liquid cyclone method was upgraded to create the gossypol separation process for air classification. Despite being financially more practical and having benefits over the liquid cyclone method, the air classification approach was never employed for production to commercial (Decossas *et al*., 1982) [8]. Gossypol concentrations in cottonseed kernels have also been evaluated as being reduced under heat and pressure settings (Gribbins, 1951) [16]. Gossypol concentrations in CSM were reduced by 91.1% after pressure cooking (Gad & El-Zalaki, 1980) [12]. Since the kernels and oil contained fewer proteins and fatty acids, this method had a limited impact on CSM detoxification. Later, degossypolization was also done via supercritical CO2 extraction. Using supercritical CO2 extraction, only 0.045% of cottonseed oil contains gossypol (Bhattacharjee, 2007) [3]. The ideal method is supercritical CO2 extraction since it is more effective, requires less time for extraction, and requires less refining because the amount of gossypol in the oil is reduced. Recent studies have shown that anti-nutritional components from various plant-based sources may be effectively reduced by gamma and electron irradiation (Fatehi *et al*., 2020; Nayefi *et al*., 2014) [11, 25]. According to Bahraini *et al*. (2017) [2], gamma and electron radiation (10, 20 and 30 kGy doses) had a significant impact on the protein quality, chemical composition, and digestibility of protein obtained from CSM. Table 1 showed the various physical methods used for removal of gossypol.

Table 1: Methods of removal of gossypol from cottonseed

Material used	Method	Conditions	Percentage of gossypol removed (%)	References
Cottonseed flakes	Chemical	Aqueous butane (90%)	79.54	Dechgary *et al*., (1952)
Cottonseed meal	Chemical	Iso-hexane+Ethanol (75+25%)	89.3%	Kuk and Hron (1998)
Cottonseed meal	Chemical	Acidic ethanol	95.7	Pelitire *et al*., (2014) [26]
Cottonseed	Chemical	Mixture of butane, ethanol and water	94.73	Singh *et al*., (2019) [31]
Cottonseed meal	Physical	Gamma and electron radiation	59.13	Nayefi *et al*., 2014 [25]
Cottonseed meal	Physical	Pressure cooking	91.1	Gad and El-Zaki (1980)
Cottonseed meal	Biological	Bacillus subtilis GH38 was used and fermentation was done at 39 °C at pH 7.5 for 72 h	78.86	Zhange *et al*., (2018)
Cottonseed meal	Biological	*Candida tropicalis* ZD-3 *Sacromyces cerevisae* and *Aspergillus niger*	95.57, 88.51 and 85.61	Zhange *et al*., (2006)
Cottonseed meal	Biological	*Candida tropicalis*	86.18	Khalaf and Meleigy (2008)
Cottonseed meal	Biological	*Sacromyces cerevisae*	88.51	Zhange *et al*., (2007)

Chemical Methods

The solution of CSM in a liquid solvent enables close contact between gossypol and the solvent. The efficacy of the procedure, nontoxicity, reusability, and low cost all influence the choice of solvent to be used (Gribbins, 1951; Smith, 1971) [16, 32]. The good gossypol extraction efficiency with extremely cheap economic inputs is one of the advantages of the solvent extraction technology.

It is important to note that numerous factors can affect the extraction procedure, including particle size, medium type, moisture content, solvent-to-seed ratio, temperature and time. (Zhang, *et al*., 2018) [34]. Gossypol may be extracted or removed from CSM that has had lysine added, which can improve the protein quality and increase the availability of lysine (Gadelha *et al*., 2014; Saki *et al*., 2012) [13]. When it attaches to the lysine in a protein, gossypol transforms into a bound compound, which acts as a toxic agent in CSM. Arginine and lysine segments of the meal protein can be bound by Gossypol through covalent interactions with the epsilon amino groups (Gadelha *et al*., 2014) [13]. It is necessary to perform further detoxification in order to avoid the denaturement of the protein caused by the attaching gossypol protein complex, which turns the isolated CSM a dark brownish-black color (Berardi & Frampton, 1957) [6]. Due to the binding of proteins to gossypol, solvent extraction of CSM at high temperatures is reported to reduce the nutritional value of CSP (Hron *et al*., 1987) [19]. According to Harris *et al*. (1949) [8], many advantageous by-products of CSM, including gossypol may be extracted and used as commercial items. As extraction solvents, light paraffinic petroleum fractions like octane, pentane and hexane are widely utilized. Among nonpolar solvents, hexane has proven to be more effective than polar solvents. Mixed solvents such commercial hexane and ethanol (Liu *et al*., 1981) [23], acetone-hexane (Kuk *et al*., 2005) [21], acetone, cyclohexane, and water and methylene chloride and ethanol have all been used in the past to extract or remove gossypol from CSM. Trichloroethylene has been investigated as a potential remedy to lessen the amount of free gossypol while generating the least amount of protein denaturation (Arnold & Juhl, 1955) [1]. Rao and Arnold (1958) extracted gossypol from cottonseeds using ethanol as a solvent in the pilot plant studies. They used four different ethanol concentrations (91.5%, 95.4%, 98%, and 99.9%) and three different temperatures (65 °C, 70 °C, and 78.3 °C) for extraction times ranging from 10 to 100 minutes. Using ten different solvents, Dechary *et al*. (1952) recovered gossypol from cottonseeds. With various solvent combinations, the percentage of free gossypol removed ranged from 7.27% for isopropanol to 79.54% for 90% aqueous butanone. Several solvent combinations eliminated free gossypol in variable amounts, from 7.27% for isopropanol to 79.54% for 90% aqueous butanone. While aqueous butanone (95%) and aqueous dioxane (90%) were both capable of eliminating 52.72% of Chlorine-substituted hydrocarbons were the least effective at removing the free gossypol from the flakes. When butanone-water pairs were used as solvent vents, the extraction temperature and amount of moisture in the extraction system had an impact on the extraction rate of gossypol. This was due to the fact that the flakes swelled as a result of the extraction system's higher moisture content, which reduced the effectiveness of solvent extraction.

Biological Methods

Researchers have devised a variety of physical and chemical deglossypolization techniques, however these techniques have a number of drawbacks. These techniques produce feed with low feed palatability, poor protein quality, and low active vitamin content while wasting a lot of energy (Zhang *et al*., 2018) [34]. When degossypolizing chemically from CSM, the leftover solvent is challenging to remove. Both ruminants and non-ruminants may be harmed by this leftover solvent. During the recovery of oil from CSM, low temperatures and a quick processing time are required to maintain protein quality and a manageable level of free gossypol. The amount of lysine in the protein was frequently decreased at higher temperatures. In order to prevent free gossypol from entering animal systems, a method for eliminating it must be created. Free gossypol can be broken down by bacteria belonging to Candida, Torulopsis, Aspergillus, Mucor, Rhizopus, and Bacillus. Table 1 provides an overview of the detoxification of free gossypol by microorganisms under optimal conditions. As a result of using gossypol as a carbon source, CSM can be detoxified by microbes in both of the following ways: first, using gossypol reduces the amount of total gossypol in the CSM; second, by converting free gossypol into bound gossypol, the fermentation process reduces the overall toxicity of the CSM. Microbial detoxification of CSM can increase the amount of proteins and amino acids present in addition to helping it to fulfill the necessary safety criteria. The levels of free and bound gossypol in the fermented samples were considerably lower than in the control (uninoculated) samples. Cow rumen fluid included a strain of recognized and described Bacillus subtilis that was later found to be responsible for the biodegradation of gossypol (Kumar *et al*., 2021) [22]. A study was conducted to evaluate the quality of CSM through short-term fermentation (4 days) and long-term fermentation (14 days) using different yeast strains. It was shown that both fermentations increased the overall amounts of both essential and non-essential amino acids (16–18%), with the biggest rise occurring in the case of M. A further reduction of 17% in gossypol occurred during fermentation, which can be attributed to the destruction of gossypol's structure by enzymatic or microbial processes (Duodu *et al*., 2018) [10]. The oxidative process of polyphenolic compound biodegradation is mediated by oxygenases, hydroxylases, peroxidases, and laccases. Due to their role in converting phenolic chemicals into their oxidised counterparts, laccases released by wood-degrading fungus have drawn a lot of interest. Although the precise process causing gossypol's biological degradation is not yet known, laccase has been seen to play a role. The researchers found that a rice straw-based enzyme extract from *Pleurotus florida* that contained laccase could effectively degrade gossypol. The rate of gossypol breakdown increased as the enzyme concentration rose. The enzyme blank that included boiling extract did not degrade gossypol (Rajarathanam *et al*., 2001) [27]. Gossypol is a polyphenolic molecule that is broken down by the enzymes laccase, peroxidase, and polyphenol oxidase. On a minimum medium containing gossypol, a mixed fungal culture was established. The laccase, peroxidase, and polyphenol oxidase activities of the crude supernatant from this culture were then determined. Researchers discovered that the crude supernatant had decreased polyphenol oxidase and peroxidase activity and increased laccase activity. The specific activity of laccase was

27–35 times greater in the purified supernatant. Researchers found that residual levels of gossypol were decreased by 30% and 60%, respectively, which supports the theory that laccase plays a role in the degradation of gossypol. The author used SDS-PAGE to determine the molecular mass of laccase in order to further validate its identification. The hazardous aldehyde stretch of degraded gossypol was significantly reduced, according to an FTIR study (Kumar *et al*., 2021) [22].

Conclusion

The main objective of this study was to develop a method for reducing the gossypol content of cottonseed flour to safe levels. This would assist in reducing its toxicity, as well as increasing its nutritional value. Gossypol has been reduced or extracted from cottonseeds using a variety of separation techniques, including physical, chemical, and biological ones. Separating gossypol from CSM will result in an abundance of high-quality edible protein, which can be used both as rations for animals as well as food for humans. According to toxicological research, gossypol is lethal if present in animal feed at levels exceeding the permitted limit of 450 parts per million. Gamma irradiation is replacing conventional methods as a cutting-edge degossypolyzation physical methodology. The study provides valuable insights into the potential of using cottonseed protein isolate as an alternative to traditional protein sources in food production. Our research has shown that detoxification can improve the quality of cottonseed protein isolate, making it a viable and sustainable option for food production. Furthermore, this research has implications for the agriculture industry, as cottonseed is a byproduct of cotton farming and can be a valuable source of protein for animal feed. The findings of this study can support the establishment of a circular economy in agriculture and encourage the use of cottonseed as a sustainable source of protein. The functional and thermal characteristics of cottonseed protein isolate have been extensively studied in this study, as well as the possibility of detoxification as a means of enhancing its quality. Our results should stimulate additional study in this field and aid in the creation of sustainable methods for growing food.

References

1. Arnold LK, Juhl WG. The reduction of free gossypol in cottonseed flakes during solvent extraction. Journal of the American Oil Chemists' Society. 1955**;**32(3):151-152.
2. Bahraini Z, Salari S, Sari M, Fayazi J, Behgar M. Effect of radiation on chemical composition and protein quality of cottonseed meal. Animal Science Journal. 2017;88(9):1425-1435.
3. Bhattacharjee P, Singhal RS, Tiwari SR. Supercritical carbon dioxide extraction of cottonseed oil. Journal of Food Engineering, 2007;79(3):892-898.
4. Boatner CH, Hall CM, Merrifield AL. U.S. Patent No. 2,482,141. Washington, DC: U.S. Patent and Trademark Office; c1949.
5. Cai Y, Xie Y, Liu J. Glandless seed and glanded plant research in cotton. A review. Agronomy for sustainable development. 2010;30:181-190.
6. Castillon Berardi L, Frampton VL. Note on gossypol and its relation to color fixation in cottonseed oil. Journal of the American Oil Chemists' Society. 1957;34(8):399-401.
7. Dechary JM, Kupperman RP, Thurber FH, Altschul AM. Removal of gossypol from cottonseed by solvent extraction procedures. Journal of the American Oil Chemists' Society. 1952;29(8):339-341.
8. Decossas KM, Kadan RS, Spadaro JJ, Ziegler GM, Freeman DW. Edible cottonseed flour by air classification of glanded seed: Cost analysis. Journal of the American Oil Chemists Society. 1982;59:488-494.
9. Zhang Y, Zhang Z, Dai L, Liu Y, Cheng M, Chen L. Isolation and characterization of a novel gossypol-degrading bacteria Bacillus subtilis strain Rumen Bacillus Subtilis. Asian-Australasian journal of animal sciences. 2018;31(1):63.
10. Duodu CP, Adjei-Boateng D, Edziyie RE, Agbo NW, Owusu-Boateng G, Larsen BK, Skov PV. Processing techniques of selected oilseed by-products of potential use in animal feed: Effects on proximate nutrient composition, amino acid profile and antinutrients. Animal Nutrition. 2018;4(4):442-451.
11. Fatehi P, Alamouti AA, Behgar M, Norouzian MA. Effect of electron irradiation on some physical, chemical and digestion properties of pistachio by-products. Radiation Physics and Chemistry. 2020;174:108921.
12. Gad TE, El-Zalaki EM. Effect of various pressure cooking conditions on gossypol content of cottonseed meal and oil. Fette, Seifen, Anstrichmittel. 1980;82(11):450-453.
13. Gadelha ICN, Fonseca NBS, Oloris SCS, Melo MM, Soto-Blanco B. Gossypol toxicity from cottonseed products. The Scientific World Journal; c2014.
14. Gardner HK, Hron RJ, Vix HLE. Removal of pigment glands (gossypol) from cottonseed. Cereal Chemistry. 1976;53:549-560.
15. Gemechu FG. Embracing nutritional qualities, biological activities and technological properties of coffee by-products in functional food formulation. Trends in Food Science & Technology. 2020;104:235-26.
16. Gribbins GH. The reduction of free gossypol in cottonseed by pressure cooking. Journal of the American Oil Chemists' Society. 1951;28(2):41-45.
17. Zhang WJ, Xu ZR, Sun JY, Yang X. Effect of selected fungi on the reduction of gossypol levels and nutritional value during solid substrate fermentation of cottonseed meal. Journal of Zhejiang University. Science. B. 2006;7(9):690.
18. Harris WD, Hayward JW, Lamb RA. Isopropanol as a solvent for extraction of cottonseed oil. II. Separation of purified oil from miscella. Journal of the American Oil Chemists' Society. 1949;26(12):719-723.
19. Hron Sr, RJ, Koltun SP, Pominski J, Abraham G. The potential commercial aspects of gossypol. Journal of the American Oil Chemists' Society. 1987;64(9):1315-1319.
20. Khalaf MA, Meleigy SA. Reduction of free gossypol levels in cottonseed meal by microbial treatment. Int. J Agric Biol. 2008;10(2):185-190.
21. Kuk MS, Tetlow R. Gossypol removal by adsorption from cottonseed miscella. Journal of the American Oil Chemists' Society. 2005;82(12):905-909.
22. Kumar M, Tomar M, Punia S, Grasso S, Arrutia F, Choudhary J, *et al*. Cottonseed: A sustainable contributor to global protein requirements. Trends in Food Science & Technology. 2021;111:100-113.
23. Liu FK, Jou SY, Jung LY. A new method of detoxification of cottonseed by means of mixed solvent extraction. Journal of the American Oil Chemists'

Society. 1981;58(2):A93-A96.
24. Manoj K, Mageshwaran V, Sujata S, D'Souza Charlene AN, Sharmila P, Archana M, *et al*. Value addition of cottonseed meal for feed industries by degossypolization employing papain and solvent. Research Journal of Biotechnology. 2019;14:11.
25. Nayefi M, Salari S, Sari M, Behgar M. Treatment by gamma or electron radiation decreases cell wall and gossypol content of cottonseed meal. Radiation Physics and Chemistry. 2014;99:23-25.
26. Pelitire SM, Dowd MK, Cheng HN. Acidic solvent extraction of gossypol from cottonseed meal. Animal Feed Science and Technology. 2014;195:120-128.
27. Rajarathnam S, Shashirekha MN, Bano Z. Biodegradation of gossypol by the white oyster mushroom, Pleurotus florida, during culturing on rice straw growth substrate, supplemented with cottonseed powder. World Journal of Microbiology and Biotechnology. 2001;17:221-227.
28. Rao RK, Arnold LK. Alcoholic extraction of vegetable oils. V. Pilot plant extraction of cottonseed by aqueous ethanol. Journal of the American Oil Chemists Society. 1958;35:277-281.
29. Rathore KS, Pandeya D, Campbell LM, Wedegaertner TC, Puckhaber L, Stipanovic RD, *et al*. Ultra-low gossypol cottonseed: selective gene silencing opens up a vast resource of plant-based protein to improve human nutrition. Critical Reviews in Plant Sciences. 2020;39(1):1-29.
30. Saki AA, Pournia K, Tabatabaie MM, Zamani P, Haghighat M, Salary J. Effects of cottonseed meal supplemented with lysine and enzyme (*Hydroenzyme* XP) on egg quality and performance of laying hens. Revista Brasileira de Zootecnia. 2012;41:2225-2231.
31. Singh S, Sharma SK, Kansal SK. Extraction of gossypol from cottonseed. Review in Advance Science and Engineering. 2015;4:301–318.
32. Smith AK. Practical considerations in commercial utilization of oilseeds. Journal of the American Oil Chemists' Society. 1971;48(1):38-42.