

Standardization of Infrared Pre-treatment Process for Detoxification of Cottonseed Meal and Enhancement of Protein Extraction

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ABSTRACT

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Cottonseed meal (CSM) is a protein-rich by-product whose utilization is restricted by the presence of toxic free gossypol. This study optimized infrared (IR) pre-treatment conditions to reduce free gossypol while preserving protein quality for efficient extraction. CSM was treated at 50–120 °C for 1–5 min under controlled conditions and analyzed for free gossypol, total gossypol, and protein content. Free gossypol decreased progressively up to 70 °C, reaching a minimum of 0.036 % at 70 °C for 5 min, but increased at higher temperatures (0.111 % at 120 °C), indicating disruption of protein–gossypol complexes. Total gossypol slightly decreased up to 70 °C (≈1.21 %) and increased thereafter (≈2.01 % at 120 °C). Protein content improved significantly at moderate temperatures, with maximum extractable protein (≈60–65 %) at 70 °C for 2–3 min, whereas severe heating (≥90 °C) caused substantial protein denaturation. Overall, IR pre-treatment at 70–80 °C for 2–5 min was identified as optimal for effective detoxification while maintaining protein integrity, offering a rapid and scalable strategy for cottonseed protein isolation and value-added applications.

Introduction

Protein is essential for metabolism, growth, and tissue repair, with a recommended intake of approximately 0.8 g/kg body weight. However, global nutrient deficiencies, environmental concerns, and a rapidly increasing population have intensified the demand for sustainable plant-based protein sources. Oilseed meals, the by-products of edible oil extraction, are underutilized

despite containing 15–50% protein and offering significant potential for waste reduction and nutritional enhancement (1, 2, 3). Plant proteins derived from such by-products are increasingly recognized as viable alternatives to animal proteins (4, 5, 6).

Cotton (*Gossypium hirsutum* L.) is cultivated in more than 80 countries and serves as both a major fiber and oilseed crop (7). Cottonseed contains about 16–20% oil,

and its processing produces approximately 45–50% cottonseed meal as a protein-rich by-product. (8). Despite its nutritional value, the utilization of CSM in human food is limited because of the presence of gossypol, a toxic polyphenolic compound that binds to the ϵ -amino group of lysine, reducing protein availability. Regulatory standards of Food and Drug Administration (1974) and the Food and Agriculture Organization (2004) recommend that free gossypol should not exceed 0.045% for safe human consumption (9). In India, the Food Safety and Standards Authority of India (2011) allows up to 0.06% free gossypol and 1.2% total gossypol on a dry weight basis.

Various detoxification methods have been studied to reduce gossypol in cottonseed meal, including physical, chemical, and biological treatments (10, 11, 12). Although chemical methods are effective in reducing gossypol, they may cause problems such as chemical residues, environmental concerns, and high processing costs (13, 14, 15). In addition, electromagnetic techniques such as gamma irradiation, electron beam treatment, and UV/IR radiation have also been explored for detoxification of agricultural products (16, 17, 18, 19).

Among these approaches, infrared (IR) radiation has been widely applied in food processing for heating, drying, and roasting due to its rapid and energy-efficient heat transfer. However, its application for gossypol detoxification in oilseeds remains relatively underexplored. Infrared heating can generate uniform internal heating and promote thermal reactions that facilitate the conversion of free gossypol into less toxic bound forms while simultaneously improving protein availability. Therefore, investigating infrared pre-treatment as a rapid, energy-efficient and environmentally friendly detoxification method offers significant potential to transform cottonseed meal into a safe and sustainable protein source for human nutrition and value-added food applications.

Materials and Methods

Cottonseed Meal (CSM) used in the present study was obtained from Quality Evaluation Unit ICAR- CIRCOT, Agricultural Research Station, Dharwad (Karnataka), India.

Apparatus and equipment: Soxhlet apparatus, Infrared dryer, grinder, and digital weighing balance, Kjeldhal

Automatic Nitrogen Distillation System, UV Spectrophotometer, Chemicals (Analytical grade).

Infrared Pre-treatment: For the experiments, the infrared dryer with 2.5 μm to 3 μm wavelength of radiation. Range of temperature was 50 °C to 100 °C. Infrared heat source was quartz infrared. The meal was exposed to infrared radiation at different wavelength, and time of pre-treatment.

Sample preparation

The initial moisture content of the cottonseed meal (CSM) ranged from 7–8% (wet basis). Preliminary trials were conducted at temperatures between 40 °C and 100 °C with varying pre-treatment durations. Increasing temperature and time consistently reduced free gossypol levels and enhanced protein content. However, temperatures above 80 °C led to a rise in free gossypol beyond safe limits and a decline in protein levels. Infrared treatment at 90 °C resulted in burning and charring of the meal. Based on these findings, pre-treatment conditions were standardized at 50 °C to 120 °C for durations of 1 to 5 minutes. The treated CSM was then passed through a 50- mesh sieve to ensure uniform particle size for subsequent analysis.

Protein Extraction of Cotton seed protein isolate

Cottonseed protein was extracted from infrared pre-treatment cottonseed meal. Protein extraction from both the meals was carried out using combined salt-alkaline extraction process. Kumar *et al.*, (2022) Initially, meal was added into salt-alkali solution having potassium hydroxide (KOH) (0.1M/L) sodium chloride salt (NaCl) (01.5 M/L) and sodium sulphite (Na₂SO₃) in a ratio of 1.33 (meal: solvent) The pH of solution was noted down. In order to solubilize the maximum amount of protein present in meal, the solution containing meal was shaken in an orbital shaker for 147.5 min (2 h 27.5 min) for extraction. After this, solution was centrifuged at 27000g for 15 min at 4 °C. The supernatant was collected for isoelectric precipitation of protein. The pH of the supernatant was reduced using pH meter to isoelectric point i.e., 4.5 by adding citric acid powder to induce precipitation of protein and kept overnight at 4°C. Protein pellet was obtained by centrifuging precipitated protein solution at 27000 g for 15 min at 4°C followed by ethanol washing and water washing of the obtained protein pellet. Finally, the washed protein pellet was lyophilized.

Experimental design

In this study, effect of infrared parameters (infrared temperature and infrared time) on crude protein and reduction of gossypol content in CSM were broadly investigated to obtain the highest level of crude protein and lowest limit of free and total gossypol,

Free gossypol content

Infrared treated cottonseed was measured according to AOCS official method number (Ba 7–58) (24) using UV Spectrophotometer (UV-1700 Pharma Spec, Shimadzu Corp. Japan). The gossypol content was calculated with the following formula:

$$\text{Corrected Absorbance} = [\text{Absorbance of solution B} - \text{Absorbance of solution A}]$$

$$G = \text{Corrected absorbance} \times \text{calibration factor}$$

$$\text{Free Gossypol}(\%) = \frac{5G}{W \times V}$$

Total gossypol (TG) content

Total gossypol (TG) content was estimated by following protocol given by AOCS official method number (Ba 8-78) (25). Absorbance values was taken through UV Spectrophotometer.

$$\text{Corrected Absorbance} = [\text{Absorbance of solution B} - \text{Absorbance of solution A}]$$

$$G = \text{Corrected absorbance} \times \text{calibration factor}$$

$$\text{Total Gossypol}(\%) = \frac{5G}{W \times V}$$

Where,

G is mg of gossypol in the sample, W is weight of sample, V is the volume of the sample.

Total protein content

The total protein content was computed using Kelpus Kjeldhal Automatic Nitrogen Distillation System (Kelpus-Supra LX VA) by the standard Kjeldahl method according to the AOAC method (26) with minor modifications. Total protein content (%) was determined using the formula given below

$$\text{Total Protein content}(\%) = \frac{\text{Titration reading} \times \text{Normality} \times 1.4007 \times 6.25}{\text{Weight of sample}}$$

Where,

1.4007 is the atomic weight of nitrogen and 6.25 is protein nitrogen conversation factor.

Results and Discussion

Effect of process variables on free gossypol of cotton seed meal

The effect of temperature and exposure time on free gossypol content is presented in Figure 4.5. It was observed that free gossypol exhibited a clear temperature-dependent response at varying infrared pre-treatment time (1-5 min). At 50°C, free gossypol levels were comparatively high, indicating that mild heating alone is insufficient for effective detoxification. As the temperature increased to 60 °C and further to 70 °C, a progressive decline in free gossypol was observed, with the lowest values recorded at 70 °C i.e. 0.036-0.046 %. This reduction suggests that moderate thermal treatment promotes either the binding of gossypol to proteins and other seed components or its degradation. A slight increase in free gossypol occurred at 80 °C, followed by a pronounced rise at 90 °C and above, with values eventually reaching around 0.105–0.111 % at 120 °C. The non-linear trend was observed and indicated the presence of an optimum temperature for detoxification, beyond which further heating disrupted previously formed complexes and released bound gossypol back into the free form. Similar temperature-dependent behaviour, characterised by an initial decrease and subsequent increase in free gossypol at higher temperatures, has been reported for heat-treated cottonseed meal in earlier studies (Zhang *et al.*, 2007; Xu *et al.*, 2022; Liu *et al.*, 2022).

From Fig.4.5, as temperature increases from 50 °C to 70 °C, free gossypol value increases upto 70 °C. It is observed that pre-treatment time exerted a significant influence on free gossypol content at each temperature. At 50 and 60 °C, free gossypol content decreases 1-5 min, indicating that prolonged heating at relatively low temperatures enhances detoxification efficiency. This time-dependent effect was more pronounced at 70 °C, where free gossypol decreased from 0.046 % at 1 min to ~0.042 g at 3 min and ~0.036 % at 5 min, confirming

that moderate temperature combined with longer residence time is particularly effective for gossypol reduction. Even at higher temperatures (90–120 °C), longer pre-treatment time slightly lower free gossypol values than less pre-treatments time, although the absolute levels remained substantially higher than those observed at 70–80°C. This pattern suggests that increased residence time favours protein–gossypol complex formation or thermal degradation. Similar result reported that extended heating under controlled conditions promotes further decline in free gossypol up to an optimum, beyond which re- liberation may occur (Zhang *et al.*, 2007; Xu *et al.*, 2022).

Overall, the results indicated that moderate heating temperatures in the range of 70–80 °C, combined with longer exposure times (4–5 min), provided the most favourable conditions for reducing free gossypol in cottonseed meal. These findings underline the importance of precisely controlling both temperature and time to maximise detoxification efficiency.

In practical terms, such thermal processing can be recommended as a simple and scalable pre-treatment step prior to more advanced detoxification methods (e.g., microbial fermentation, solvent treatment or enzymatic approaches), thereby improving the safety of cottonseed meal for feed applications and enhancing its suitability as a substrate for protein extraction and cotton seed protein isolate production.

Effect of process variables on total gossypol of cotton seed meal

The effect of temperature and exposure time on total gossypol content is presented in Figure 4.6. Across all treatment durations (1, 2, 3, 4 and 5 min), total gossypol showed a distinct temperature-dependent response. At 50 °C, total gossypol levels were relatively high (≈1.44–1.47 %), and a slight decline was observed as the temperature increased to 60 °C and further to 70 °C, where the lowest total gossypol values (≈1.22–1.27 %) were recorded depending on pre-treatment time. This initial decrease suggests that moderate heating may promote limited degradation or structural rearrangement of gossypol and related pigments, or a redistribution between solvent extractable and matrix-bound forms, resulting in a small reduction in the measured total gossypol content. However, beyond 70°C, total gossypol increased steadily with temperature. At 80 °C, values increased in the range of 1.29–1.32 %, followed by a

more rise at 90–120 °C, ultimately reaching approximately 1.97–2.01 % at 120 °C.

This non-linear trend was due the fact that, total gossypol initially decreased and then increased at higher temperatures. Higher heating temperatures promote the formation of thermally stable gossypol derivatives and protein–gossypol complexes, which are still measured in total gossypol assays, leading to an apparent increase in total gossypol content. Similar temperature-dependent behaviour, where total gossypol remains unchanged or increases despite changes in free gossypol, has been reported for heat-treated cottonseed products (Zhang *et al.*, 2007; Xu *et al.*, 2022; Liu *et al.*, 2022).

Pre-treatment time had a noticeable effect on total gossypol content at all temperatures. At lower temperatures (50–70 °C), longer heating times (4–5 min) resulted in slightly higher total gossypol values compared to shorter treatments (1–2 min), even though free gossypol decreased. This indicates that extended heating at moderate temperatures converts free gossypol into bound or modified forms, which are still measured as total gossypol.

At higher temperatures (90–120 °C), total gossypol values increased similarly for all exposure times, showing that temperature had a stronger influence than time. In this range, treatment time mainly affected how fast the changes occurred rather than the overall trend. The sharper increase in total gossypol between 80 and 120 °C suggests that intense heating promotes thermal reactions such as condensation and polymerization between gossypol and seed components, leading to higher measured total gossypol. Similar trends have been reported in earlier studies, where severe heat treatment changed the form of gossypol without reducing its total content (Zhang *et al.*, 2007; Liu *et al.*, 2022).

Overall, the results demonstrate that, free gossypol, total gossypol does not decrease substantially with increasing temperature up to 70 °C followed by a pronounced rise at higher temperatures. From a processing perspective, this underscores the need to distinguish clearly between free and total gossypol when evaluating detoxification efficiency. Optimum infrared heating conditions (around 70–80 °C for 3–5 min) free gossypol to safer levels. Further excessive increases in infrared pretreatment time and temperature increased the total gossypol which may reflect extensive thermal modification of the cottonseed matrix.

Fig.1 Process flow chart for preparation of Cotton seed meal

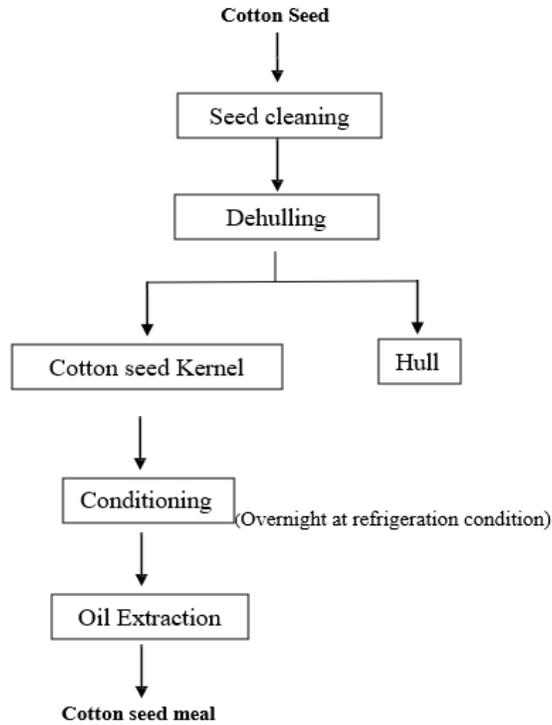


Figure.2 Effect of process variables on free gossypol of cotton seed meal

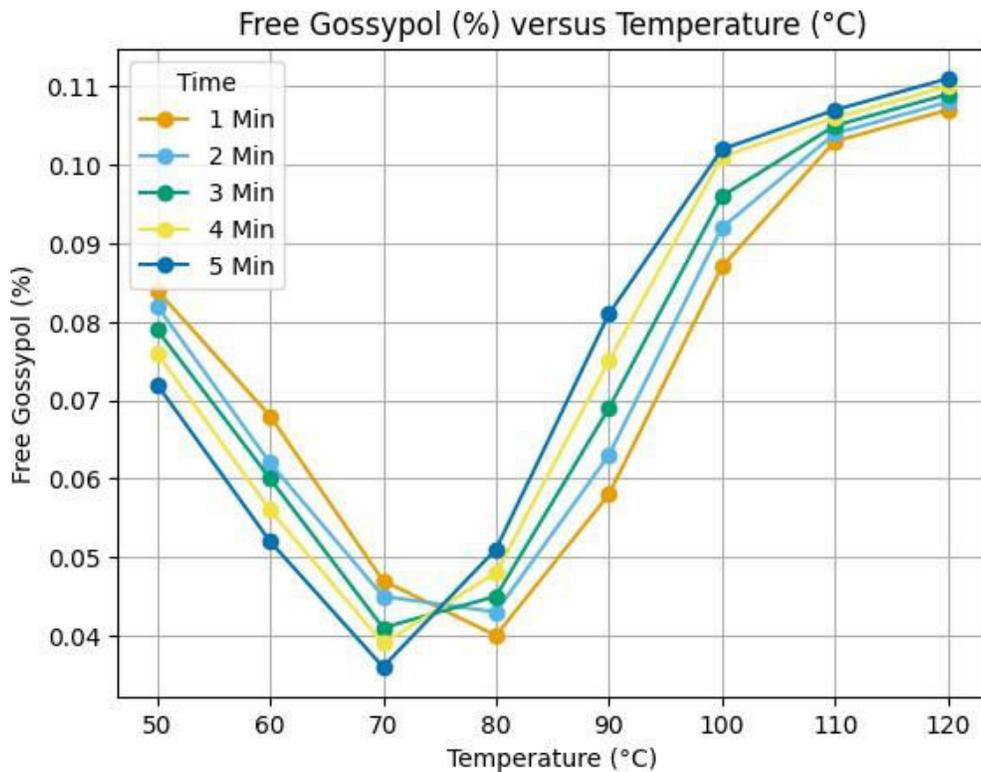


Figure.3 Effect of process variables on total gossypol of cotton seed meal

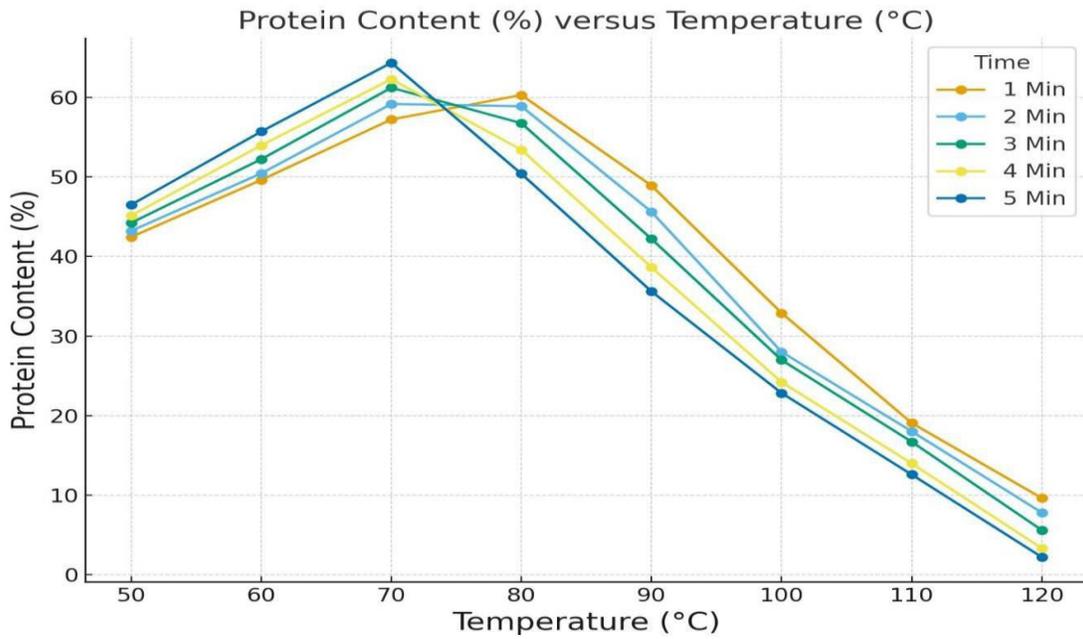


Figure.4 Effect of process variables on protein content of cotton seed meal

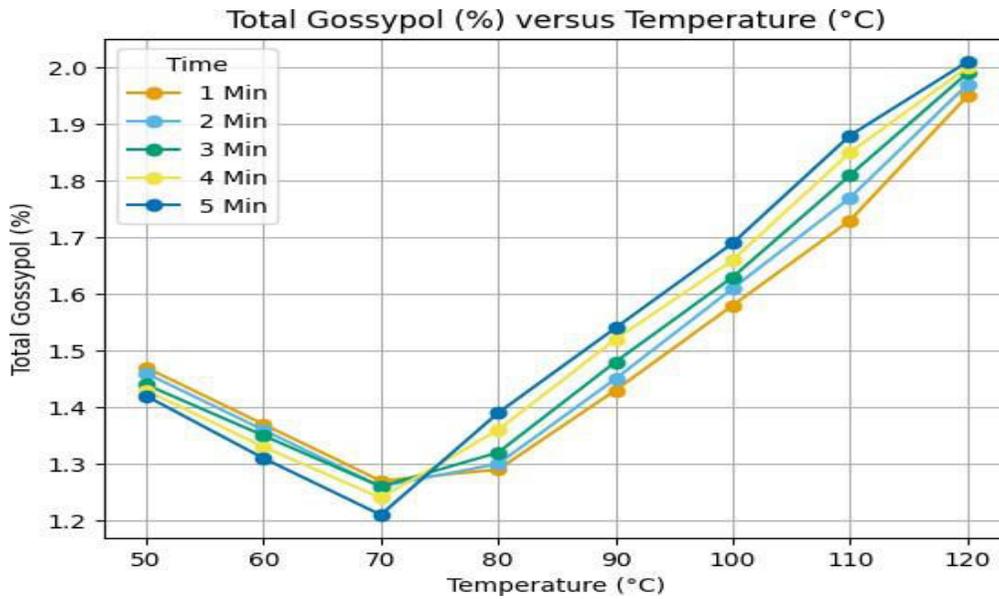


Table.1 Infrared pre-treatment for extraction of protein from cotton seed meal

Sr. No	Independent variable	Levels	Dependent Variable
1.	Infrared temperature	50°C to 120°C	Protein yield Free Gossypol Total Gossypol
2.	Time	1 to 5 Min	
Total no. of experiments = 40			

Table.2 Effect of temperature and pre-treatment time on free gossypol, total gossypol and protein content

Treatment	Temperature	Time	Free gossypol	Total gossypol	Protein Content
T1	50	1	0.0843	1.472	42.44
T2	50	2	0.0818	1.459	43.21
T3	50	3	0.0793	1.444	44.22
T4	50	4	0.0759	1.432	45.13
T5	50	5	0.0724	1.424	46.49
T6	60	1	0.0678	1.372	49.61
T7	60	2	0.0621	1.362	50.43
T8	60	3	0.0599	1.357	52.21
T9	60	4	0.0559	1.332	53.95
T10	60	5	0.0521	1.310	55.69
T11	70	1	0.0474	1.271	57.21
T12	70	2	0.0449	1.265	59.16
T13	70	3	0.0413	1.2572	61.2
T14	70	4	0.0392	1.2369	62.28
T15	70	5	0.0362	1.2103	64.34
T16	80	1	0.0401	1.2893	60.28
T17	80	2	0.0425	1.3034	58.88
T18	80	3	0.0446	1.325	56.75
T19	80	4	0.0479	1.3582	53.41
T20	80	5	0.0514	1.3873	50.39
T21	90	1	0.0578	1.4329	48.91
T22	90	2	0.0631	1.4562	45.61
T23	90	3	0.0691	1.4781	42.21
T24	90	4	0.0753	1.5198	38.64
T25	90	5	0.0812	1.5395	35.61
T26	100	1	0.0867	1.5805	32.9
T27	100	2	0.0923	1.6068	28.01
T28	100	3	0.0964	1.6342	26.99
T29	100	4	0.1007	1.6549	24.21
T30	100	5	0.1018	1.6894	22.84
T31	110	1	0.1027	1.7341	19.08
T32	110	2	0.1037	1.7701	18.03
T33	110	3	0.1044	1.8089	16.72
T34	110	4	0.1051	1.8451	14
T35	110	5	0.1063	1.8801	12.6
T36	120	1	0.1072	1.9482	9.64
T37	120	2	0.1079	1.9643	7.82
T38	120	3	0.1086	1.9847	5.62
T39	120	4	0.1094	2.0047	3.34
T40	120	5	0.11102	2.0097	2.23

Such optimised heat treatment may be recommended for detoxification or protein-isolation processes, helping to balance safety, functionality and product quality in cottonseed meal utilisation.

Effect of process variables on protein content of cotton seed meal

The effect of temperature on protein content of cottonseed meal is presented in Figure 4.7. All treatment temperature, protein content exhibited a pronounced temperature-dependent pattern. At 50 °C, protein content was relatively low (≈42–47%), indicating that mild heating alone does not sufficiently enhance protein extractability. As the temperature increased to 60 °C and further to 70 °C, a marked rise in protein content was observed for all exposure times, with maximum values of approximately 60–65% recorded at 70 °C (highest for the 2–3 min treatments). This sharp increase suggests that moderate thermal treatment promotes protein unfolding, disruption of cell structures and increased solubilisation, thereby improving the availability of protein for extraction. Similar effects of mild heating on improved protein solubility and functionality have been reported for other plant and animal protein systems by Grossmann, L. *et al.*, 2023, Limpisophon *et al.*, 2024 where controlled temperature elevation enhanced dispersibility, water-holding capacity and extraction yield. When the temperature was raised further to 80 °C, protein content either plateaued or began to decline slightly, and a progressive decrease became evident at 90–120 °C, where values dropped to below 10% at 120 °C for all roasting times. The decline at higher temperatures is consistent with heat-induced aggregation and denaturation of proteins, which reduces solubility and extractable protein yield, as documented for various legume and cereal proteins subjected to excessive thermal treatment (Li *et al.*, 2023; Limpisophon *et al.*, 2024).

Pre-treatment time is a major influencing process variable on protein content at different temperatures, protein unfolding and diffusion of soluble components. For example, at 70 °C, protein content increased from around 58–60% at 1 min to approximately 62–65% at 2–3 min and remained comparatively high at 4–5 min. This trend agrees with previous findings that short to intermediate heating periods (typically 3–9 min) at moderate temperatures improve protein solubility and hydration properties without causing severe damage to the protein network (Li *et al.*, 2023; Nguyen *et al.*,

2024). However, at elevated temperatures (90–120 °C), prolonged heating accelerated the loss of extractable protein: for a given temperature, protein content tended to be lowest at 5 min and highest at 1 min, indicating that extended exposure under severe thermal conditions intensifies aggregation, Maillard reactions and possible cross-linking with other seed constituents. Comparable time-dependent losses at high temperature have been described for plant and fish proteins where excessive heating led to irreversible denaturation, reduced solubility and deterioration of functional quality (Limpisophon *et al.*, 2024; Jiayue Tang *et al.*, 2024). Overall, the results demonstrate that moderate roasting temperatures (about 70–80 °C) combined with short to intermediate exposure times (2–3 min) provide the most favourable conditions for maximising protein content, whereas harsher treatments (≥90 °C and longer times) markedly impair protein extractability. These findings are consistent with previous reports on thermally treated plant and animal proteins and support the use of carefully controlled heat processing to enhance the nutritional and functional properties of cottonseed protein while avoiding detrimental over-processing.

In conclusion, Infrared (IR) pre-treatment effectively reduced gossypol while improving protein extractability in cottonseed meal. Free gossypol decreased to a minimum of 0.036 % at 70 °C, while protein content increased to 60–65 % under moderate heating conditions.

However, temperatures above 80 °C resulted in increased gossypol and reduced protein due to thermal denaturation. Therefore, 70–80 °C for 2–5 min was identified as the optimal infrared pre-treatment condition for efficient detoxification and improved protein utilization of cottonseed meal.

Author Contributions

Shalaka Kalamnurikar: Investigation, formal analysis, writing—original draft. Smita Khodke: Validation, methodology, writing—reviewing. Dattatreya Kadam:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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