Optimization Of Microwave Pre-Treatment Process for Detoxification of Cottonseed Meal for Protein Extraction

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***Abstract*—Cotton Seed Meal (CSM) is a protein-rich by-product of the cottonseed oil industry with significant potential for human nutrition. However, its utilization is restricted due to the presence of gossypol, a toxic polyphenolic compound. The present study investigated microwave pre-treatment as a rapid, chemical-free technique for detoxification of cottonseed meal and enhancement of protein availability. Response Surface Methodology (RSM) was employed to optimize microwave power, moisture content, and microwave time for effective reduction of free and total gossypol while maximizing protein content. The optimized conditions of the process of microwave pre-treatment were identified as 540 W microwave power, 26% moisture content, and 6 min microwave time. Under these optimized conditions, free gossypol was reduced to 0.045%, total gossypol to 1.19%, and protein content increased to 52.52%. Response surface plots illustrated the interactive effects of variables on detoxification and protein enhancement. The predicted values were validated experimentally with minimal deviation. The study demonstrates that microwave pre-treatment is an efficient and eco-friendly method for detoxifying cottonseed meal to safe regulatory limits while improving protein extractability, offering strong potential for food applications.**

***Index Terms*—Cottonseed meal, Gossypol detoxification, Microwave pre-treatment, Protein extraction**

I. 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 [1,11,12]. 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, microwave radiation is widely used in food processing for heating, drying, and sterilization, yet remains underexplored for gossypol detoxification in oilseeds. Therefore, investigating microwave pre-treatment as a rapid, eco-friendly detoxification method offers strong potential to convert cottonseed meal into a safe, sustainable protein ingredient for human nutrition.

II. 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.

2.1 Apparatus and equipment: Soxhlet apparatus, Microwave oven, grinder, and digital weighing balance, Kjeldhal Automatic Nitrogen Distillation System, UV Spectrophotometer, Chemicals (Analytical grade).

2.2 Microwave Pre-treatment

For the experiments, the microwave oven (Samsung, Model CE2977NT, 230 V/ 50 Hz, South Korea) with different power levels – 180 W, 540 W and 900 W was used. 50 grams of cotton meal sample was taken and spread evenly in a thin layer of 3 mm thickness on a glass dish. The meal was exposed to microwave radiation at varying levels of microwave powers (180W, 540W, 900W), microwave time (2min, 6 min, 10 min) of cotton seed meal.

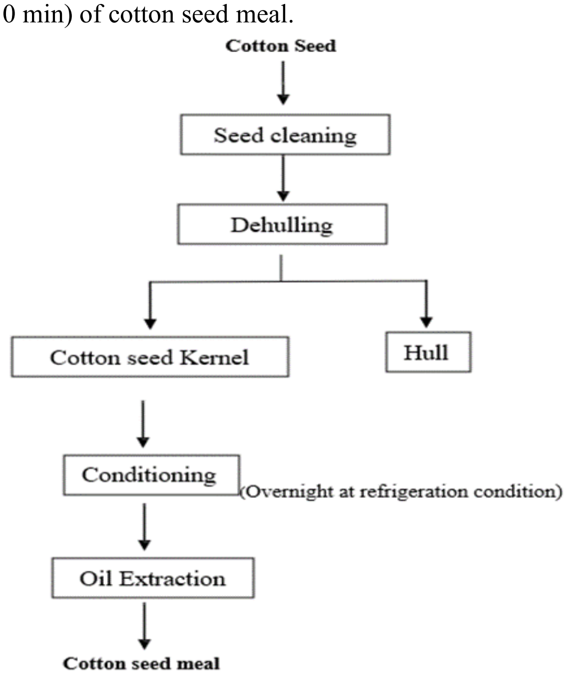

Fig 2.1: Process flow chart for preparation of Cotton seed meal

2.3 Sample preparation

The initial moisture content of the cottonseed meal was 7–8% (wet basis). At this low moisture level, microwave treatment led to burning and charring of the meal. In addition, previous studies have reported that the tough walls of pigment glands rupture more easily when the material is properly moistened [20,21,22]. Therefore, the moisture content of the meal was increased to the desired range prior to microwave pre-treatment. Preliminary trials were carried out with preconditioning moisture levels ranging from 15% to 35%. An increase in the sample's moisture content from 15% to 21% led to a progressive decline in free gossypol levels accompanied by an improvement in protein content; however, the free gossypol content still exceeded the safe limit. The trials were continued until the free gossypol content reached a safe limit while maintaining a high protein level. Moisture contents exceeding 30% caused a gradual rise in free gossypol levels, surpassing the safe threshold, and were accompanied by a decline in protein content. Accordingly, the pre-treatment moisture levels were selected as 22%, 26%, and 30%, and the seed meal was preconditioned by adjusting its moisture content to these levels (wet basis) prior to microwave treatment [23]. Alteration in moisture content to the desired levels was done by adding calculated amount of water into meal using equation (3.1), by overnight tempering in refrigerator for moisture equilibrium. Conditioned CSM was screened with a 50-mesh sieve to ensure uniform particle size for further analysis.

$$W_m = M_1 \frac{\Delta M}{100 - M_2} \qquad (3.1)$$

Were,

W_m = Amount of water to be added,

M_1 = Initial weight of sample at moisture content,

M_2=Desired moisture content, Initial moisture content

2.4 Experimental design

In this study, effect of microwave parameters (microwave power and microwave time) and pre-treatment of moisture content 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, microwave treatment process was optimized using response surface methodology (RSM) based Box-

Behnken Design (BBD). The process parameters and range of levels used for experimentation was selected to assess combined results of moisture content for pre-treatment (22, 26, and 30 %), microwave power level (180, 540, and 900 W), and microwave time (2, 6 and 10 min) using Design Expert 11 (Stat-Ease Inc, Minneapolis, USA) software. Based on the findings of initial trial factors and their levels were chosen.

Table 3.1: Optimization of process parameters for extraction of protein from cottonseed meal using Microwave pre-treatment

Sr. No	Independent Variable	Levels	Dependent Variable
1.	Microwave power	180,540,900 W	1.Protein content 2. Free Gossypol 3. Total Gossypol
2.	Moisture content	22%,26%,30%	
3.	Time	2, 6, 10 min	

Seventeen experiments trials including 5 central points were performed according to a second order box-Behnken design (BBD) with three levels and three variables. This design was selected as it fulfilled most of the requirements needed for the Optimization of process parameters using Microwave pre-treatment for extraction of protein from cottonseed meal. The quality parameters such as free gossypol, total gossypol, protein content of cotton cake/meal were determined.

2.5 Free gossypol (FG) content

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

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

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

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

2.6 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.

2.5 Total protein content

The total protein content was computed using Kelpus Kjeldhal Automatic Nitrogen Distillation System (Kelplus-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.

2.6 Validation

Microwave pre-treatment for extraction of protein from deoiled cotton cake/meal were prepared at optimum conditions of process variables *viz.*,microwave power, moisture content, pretreatment time. Further quality characteristics of response variables *viz.*, free gossypol, total gossypol and protein content were determined and verified with the predicted values of response variables given by the software.

III. RESULTS AND DISCUSSIONS

The meal used in study moisture content between 7 -8 %, crude protein content 48.28%. Before carrying out experiments, meal used as raw material was determined on basis of protein content, free gossypol (FG) and total gossypol (TG).

3.1 Effect of process variables on Free Gossypol (FG) of cottonseed meal

Microwave pre-treatment significantly reduced the free gossypol content of cottonseed meal (CSM), with values ranging from 0.044 to 0.077% in the experimental range (Table 3.2). The quadratic model was fitted with the experimental data and the statistical significance for linear, quadratic and interaction terms were analyzed for free gossypol value of cottonseed meal. Microwave power, moisture content and time had significant effect at 5% level on free gossypol content. The lack of fit F-value was non-significant for model obtained. The regression analysis was conducted to evaluate to the effect of dependent variables and likely interaction between them and to evaluate statistical significance of model in terms of coded values of variables in given equation 3.1.

Free gossypol (FG) = 0.045 - 2.900 A - 1.750 B - 0.011C + 1.950 AB +3.250 AC +1.250 BC + 0.014 A^2 + 0.014 B^2 + 0.011 C eq… (3.1)

Where A, B, C was encrypted as microwave power(W), moisture content (%) and microwave time (min) of cottonseed meal.

It was observed that microwave power was the most dominating factor to reduce free gossypol (FG) content, followed by moisture content and microwave time. The negative coefficients of the linear terms of A (microwave power), B (moisture content) and C (microwave time) in eq. (3.1) indicated that FG decreased with increase in these variables. However, the positive coefficients of the interaction terms AB, AC and BC and the quadratic terms A^2, B^2 and C^2 resulted in an increase in FG at higher levels of the variables. The presence of quadratic terms in the regression equation on FG resulted in a curvilinear nature of the response surface.

To visualize the combined effects of two variables on free gossypol content, Figure 3.1(a) shows that the free gossypol content decreased with increasing moisture content (%) and microwave power (W) up to an optimum level of 26% of moisture content and 540 W of microwave power. Beyond these levels, a further increase in moisture content and microwave power resulted in an increase in free gossypol content. This behavior may be attributed to the presence of aldehyde and hydroxyl functional groups in the free gossypol molecule, which are susceptible to heat treatment, as reported by Nicolon S. (2012).

Figures 3.2(b) and 3.2(c) shows that an increase in microwave pre-treatment time up to an optimum duration of 6 minutes resulted in a decrease in the free gossypol content of cottonseed meal; however, further increases in microwave time led to an increase in free gossypol content.

3.2 Effect of process variables on Total Gossypol content of cottonseed meal

Microwave pre-treatment of CSM resulted in a reduction in total gossypol content of cotton seed meal, which ranged from 1.027 to 1.441 % in the experimental range (Table 3.2). Microwave power, moisture content, and microwave time showed the significant effect on total gossypol at the 5% level of significance.

Total gossypol= 1.14 - 0.0047 A + 0.051 B + 0.035 C - 8.500 AB + 0.037 AC – 0.036 BC + 0.21 A^2- 0.023 B^2
+ 0.022C^2 eq… (3.2)

Where A, B, C was encrypted as microwave power, moisture content and microwave time.

It was observed from eq.3.2, that moisture content was the most influencing factor affecting total gossypol content, followed by microwave time and microwave power. The positive coefficients of the linear terms of B (moisture content) and C (microwave time) and the negative coefficient of A (microwave power) in Eq. (3.2) indicated that total gossypol increased with increasing moisture content and microwave time, while it decreased slightly with increase in microwave power within the experimental range studied. However, the negative coefficients of the interaction terms AB and BC and the positive coefficient of AC, along with the quadratic terms A^2, B^2, and C^2, contributed to additional variations in total gossypol at higher levels of these variables. The inclusion of these quadratic terms in the regression equation resulted in a curvilinear response surface for total gossypol.

The response surface through Fig. 3.3 (a-c) shows simultaneous effects of microwave power, moisture content and microwave time on total gossypol of cottonseed meal. Figures 3.3(a–c) shows that the total gossypol content of cottonseed meal increased with decreasing microwave power (A) up to 540 W, increasing moisture content (B) up to 26%, and increasing microwave treatment time (C) up to 6 min. Beyond these optimum levels, further increases in the respective process parameters resulted in a reduction in total gossypol content. However, effective degradation of total gossypol required longer microwave time. Similar observations were reported by Taghinejad- Roudbaneh et al., (2006). He stated that physical processing methods such as roasting, gamma-ray irradiation, and microwave treatment significantly reduced gossypol levels in cotton seed meal.

3.3 Effect of process variables on Protein content of cottonseed meal

Microwave pre-treatment of Cotton Seed Meal (CSM) resulted in an increase in crude protein content, ranging from 40.87 to 52.78%, at varying microwave power (180, 540, and 900 W), moisture content (22, 26, and 30% w.b.) and microwave time (2, 6, and 10 min), as shown in Table 3.2. Microwave power, moisture content, and microwave time had a significant effect on crude protein content. The highest crude protein content (52.78%) was obtained at 26% moisture content, 540 W microwave power, and a microwave time of 6 min. Overall, crude protein content increased with increasing microwave power and microwave time up to the optimum level of 540W; however, a further increase in microwave power to 900 W resulted in a reduction in crude protein content.

$$\text{Protein content} = 52.52 + 0.46\,A + 0.52\,B - 0.59\,C - 0.022\,AB - 2.0\,AC + 0.98\,BC - 2.64{*}A^2 - 7.90{*}B^2 - 1.78{*}C^2 \quad \text{eq… (3.3)}$$

Where A, B, C were noted as microwave power, moisture content and microwave time.

It was observed from eq. 3.3, that moisture content was the most influencing factor affecting protein content, followed by microwave time and microwave power. The positive coefficients of the linear terms of A (microwave power) and B (moisture content) and the negative coefficient of C (microwave time) in Eq. (3.3) indicated that protein content of cottonseed meal increased with increase in microwave power and moisture content, while it decreased with increase in microwave time within the experimental range studied. However, the negative coefficients of the interaction terms AB and AC and the positive coefficient of BC, together with the negative quadratic terms A^2, B^2 and C^2, resulted in a decrease in protein content at higher levels of the variables. The presence of these quadratic terms in the regression equation on protein content resulted in a curvilinear nature of the response surface.

In the present study, microwave pre-treatment increased crude protein content as compared to the untreated meal. Similar observations were reported by Sadeghi and Shawrang (2005) and they reported that microwave treatment of canola meal for microwave power 800W and microwave time 6 minutes enhanced crude protein in vitro digestibility [31].

Optimization and validation of the model

The numerical optimization was carried out by putting the values of independent variables within the experimental range and by setting desirable goals for responses. The graphical optimization was carried out by superimposition of contours to find the range of process parameters in which the values of responses given by numerical optimization. It is evident from Fig. 3.4 that the predicted values of all responses lay well within the range. The experiments for Microwave pre-treatment for extraction of protein from deoiled cottonseed meal were conducted at optimum process conditions viz., microwave power (W) = 540W, moisture content (%) = 26%, time (min) = 6 min for testing the adequacy of model equations for predicting the response values. The observed experimental values (average mean values of 3 replication) and values predicted by the equations of the model are presented in Table 3.3.

Table 3.3: Predicted and experimental values of responses at optimum process conditions

Responses	Predicted value	Experimental value	CV (%)
Free gossypol(g)	0.045	0.044	1.59
Total gossypol (%)	1.19	1.12	4.29
Protein content (%)	52.52	52.48	0.05

IV. CONCLUSION

The optimum conditions were identified as microwave power of 540 W, moisture content of 26%, and microwave time of 6 minutes. Under these conditions, free gossypol was reduced to 0.045%, total gossypol to 1.19%, while protein content increased to 52.52%. Microwave pre-treatment was found to be an effective, rapid, and eco-friendly technique for detoxification of cottonseed meal and enhancement of protein yield.

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Table 3.2 Effect of different process variables yon anti-nutritional factor and protein content of cotton seed meal

Sr. No.	Process variables			Free Gossypol (%)	Total gossypol (%)	Protein content (%)
	A	B	C			
1	180	22	6	0.077	1.31	42.21
2	900	22	6	0.070	1.232	43.12
3	180	30	6	0.071	1.441	40.87
4	900	30	6	0.0718	1.329	41.69
5	180	26	2	0.076	1.421	48.182
6	900	26	2	0.061	1.255	49.18
7	180	26	10	0.072	1.416	47.02
8	900	26	10	0.070	1.399	48.01
9	540	22	2	0.071	1.027	44.76
10	540	30	2	0.064	1.187	42.12
11	540	22	10	0.073	1.17	41.6
12	540	30	10	0.071	1.186	42.87
13	540	26	6	0.044	1.15	52.78
14	540	26	6	0.046	1.11	52.48
15	540	26	6	0.047	1.171	52.54
16	540	26	6	0.045	1.15	52.46
17	540	26	6	0.044	1.14	52.32

Table 3.4 Analysis of variance (ANOVA) showing effect of process variables on the free gossypol values of cotton seed meal

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	2.483E-003	9	2.759E-004	73.53	< 0.0001*	Significant
A-MP	6.728E-005	1	*6.728E-005*	17.93	0.0039	
B-MC	2.178E-005	1	2.178E-005	5.81	0.0468	
C-TIME	2.450E-005	1	2.450E-005	6.53	0.0378	
AB	1.521E-005	1	1.521E-005	4.05	0.0839	
AC	4.225E-005	1	4.225E-005	11.26	0.0122	
BC	6.250E-006	1	6.250E-006	1.67	0.2378	
A^2	7.816E-004	1	*7.816E-004*	208.36	< 0.0001	
B^2	*7.816E-004*	1	*7.816E-004*	208.36	< 0.0001	
C^2	*5.025E-004*	1	*5.025E-004*	133.96	< 0.0001	
Residual	2.626E-005	7	3.751E-006			
Lack of Fit	1.946E-005	3	6.487E-006	3.82	0.1143	Not significant
Pure error	6.800E-006	4	1.7E-006			
Core total	2.509E-006	16				
R^2	0.989					
Adjusted R^2	0.976					
Predicted R^2	0.871					

Table 3.5 Analysis of variance (ANOVA) showing effect of process variables on the total gossypol values of cotton seed meal

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	0.24	9	0.027	81.89	< 0.0001	Significant
A-MP	0.017	1	0.017	52.52	0.0002	
B-MC	0.020	1	0.020	61.61	0.0001	
C-TIME	9*.870E-003*	1	9.870E-003	29.81	0.0009	
AB	2.890E-004	1	2.890E-004	0.87	0.3813	
AC	*5.550E-003*	1	5.550E-003	16.76	0.0046	
BC	*5.184E-003*	1	5.184E-003	15.65	0.0055	
A^2	0.18	1	0.18	544.95	< 0.0001	
B^2	*2.271E-003*	1	2.271E-003	6.86	0.0345	
C^2	*1.951E-003*	1	1.951E-003	5.89	0.0456	
Residual	2.318E-003	7	3.312E-004		< 0.0001	
Lack of Fit	*3.453E-004*	3	1.151E-004	0.23	0.0002	Not significant
Pure error	1.973E-003	4	4.932E-004			
Core total	0.25	16				
R^2	0.990					
Adjusted R^2	0.978					
Predicted R^2	0.965					

Table 3.6 Analysis of variance (ANOVA) showing effect of process variables on protein content values of cotton seed meal

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	336.52	9	37.39	712.79	< 0.0001	Significant
A-MP	1.73	1	1.73	32.94	0.0007	
B-MC	2.14	1	2.14	40.84	0.0004	
C-TIME	2.81	1	2.81	53.58	0.0002	
AB	2.025E-003	1	2.025E-003	0.039	0.8498	
AC	1.600E-005	1	1.600E-005	3.050E-004	0.9866	
BC	3.82	1	3.82	72.86	< 0.0001	
A^2	29.38	1	29.38	560.06	< 0.0001	
B^2	262.91	1	262.91	5011.93	< 0.0001	
C^2	13.29	1	13.29	253.32	< 0.0001	
Residual	0.37	7	0.052			
Lack of Fit	0.25	3	0.085	2.99	0.1585	Not significant
Pure error	0.11	4	0.028			
Core total	336.89	16				
R^2	0.998					
Adjusted R^2	0.997					
Predicted R^2	0.987					

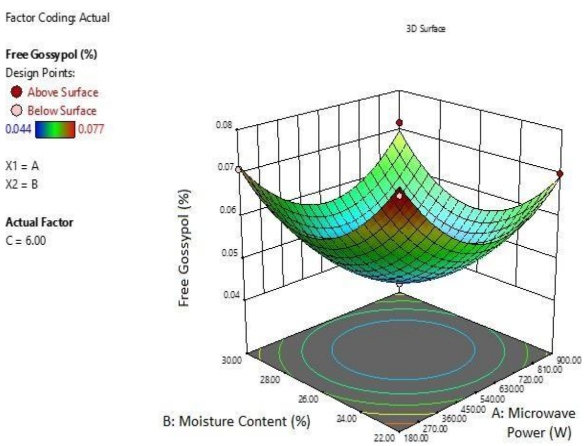

Fig. 3.1(a): Effect of Microwave power(A) and Moisture content (B) on Free gossypol (g) values of cotton seed meal at constant Microwave time

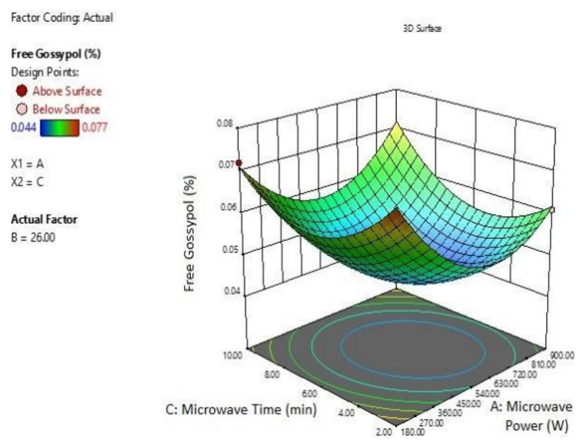

Fig.3.1(b): Effect of Microwave power(A) and Microwave time (C) on Free gossypol (g) values of cotton seed meal at constant Moisture content

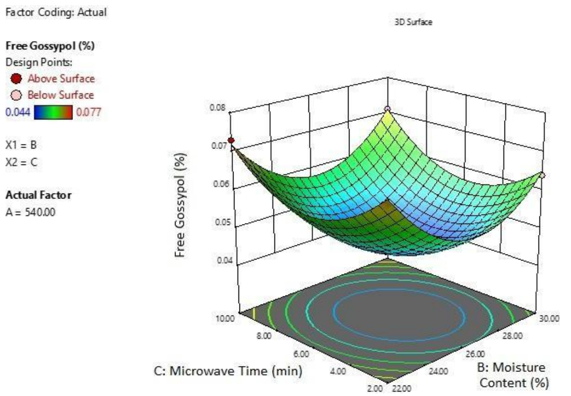

Fig.3.1(c): Effect of Moisture content (B) and Microwave time (C) on Free gossypol (g) values of cotton seed meal at constant Microwave power

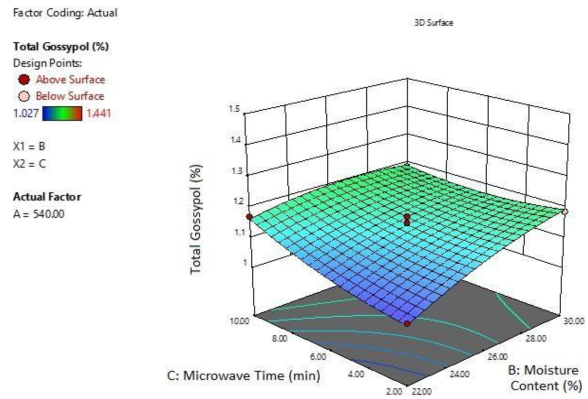

Fig.3.2(c): Effect of Moisture content (B) and Microwave time(C) on Total gossypol (g) values of cotton seed meal at constant Microwave power

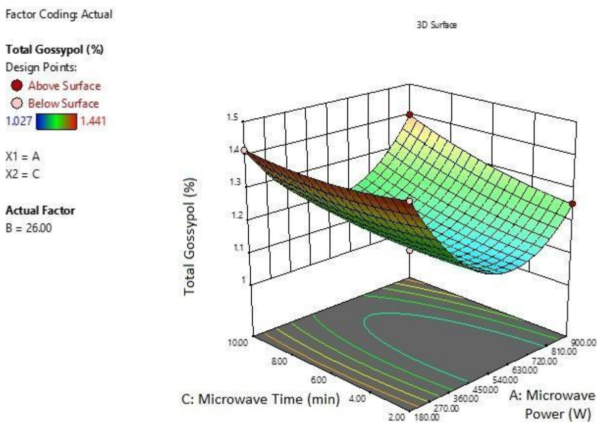

Fig.3.2(a): Effect of Microwave power (A) and Microwave time (C) on Total gossypol (g) values of cotton seed meal at constant Moisture content

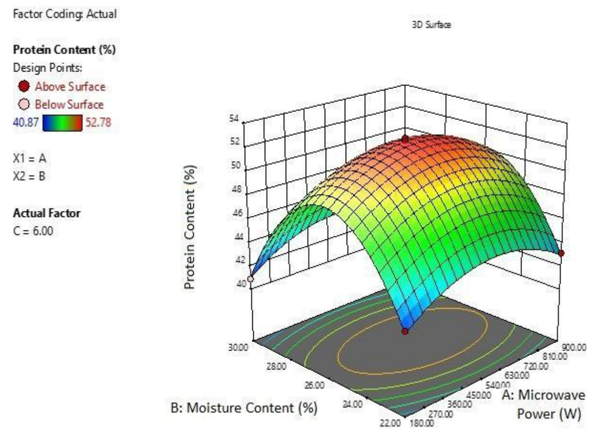

Fig 3.3 (a) Effect of Microwave power(A) and Moisture content (B) on Protein content (%) values of cotton seed meal at constant Microwave time

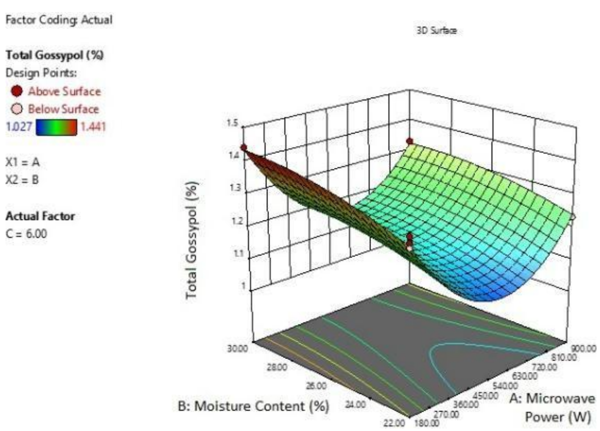

Fig.3.2(b): Effect of Microwave power (A) and Moisture content (B) on Total gossypol (g) values of cotton seed meal at constant Microwave time

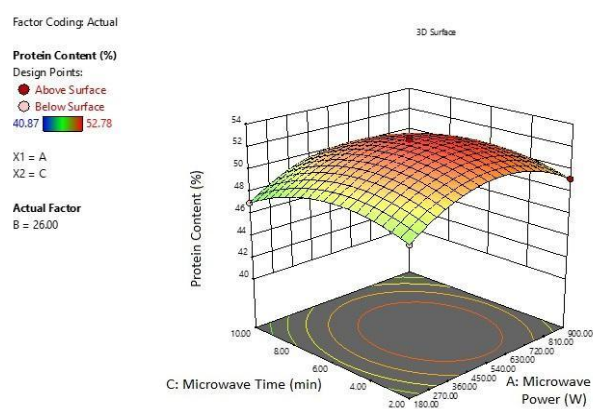

Fig 3.3 (b) Effect of Microwave power(A) and Microwave time (C) on Protein content (%) values of cotton seed meal at constant Moisture content

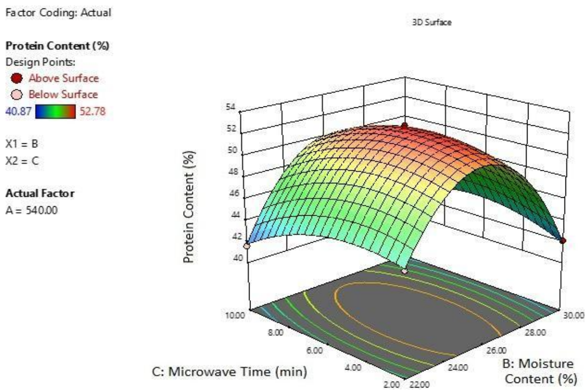

Fig 3.3 (c) Effect of Moisture content (B) and Microwave time (C) on Protein content (%) values of cotton seed meal at constant Microwave power

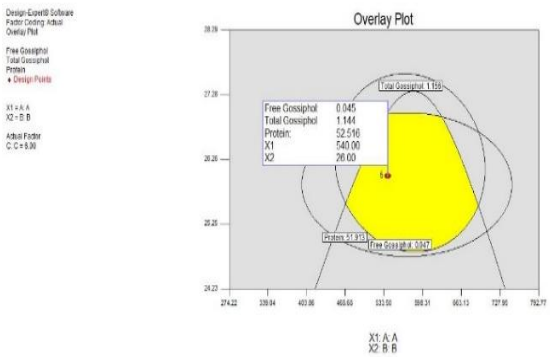

Fig 3.4 Contour plots for Microwave power(A) and Moisture content (B) on response variables