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RESEARCH PAPER

Effects of enzymatic, sprouting and solvent methods on free and total gossypol reduction, oil content, flour yield and crude protein content

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Abstract : Use of cottonseed as protein source for food application and monogastric animals are restricted due to the presence of polyphenolic toxic content known as gossypol. Reduction of gossypol and improving crude protein content in cottonseed flour may increase its utility for feed and food industries. The present study aims to analyze the influence of three individual methods *viz.,* sprouting method, enzymatic method and solvents method on free and total gossypol content, crude protein content, oil content and flour yield. Enzymatic treatments were performed using culture candida tropicalis, saccharomyces cerevisiae and combinations of both whereas for solvent treatments four different solvents namely acetone, ethanol, methanol and iso-propanol with their four different levels (70-100%) were applied. Results of this study revealed that 70% aqueous acetone showed maximum free gossypol reduction (97%), crude protein content (65.6%), oil content 31.3 % and 57% flour yield was observed. However, total gossypol reduction was only 51%. Other side, enzymatic treatment based on candida tropicalis found more effective in terms of total gossypol reduction (73%), however crude protein content was recorded only 39.4%.

Key Words : Aqueous acetone, Gossypol, Crude protein, Solvent, Cottonseed, Culture

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INTRODUCTION

India is the leading producer of cotton in the world. In the year 2018-19 the total cotton production of India was 5.77 million metric tons. Cottonseed constitutes two third portion of the cotton and is the main by-product of cotton obtained after ginning. Cottonseed is mainly used for cottonseed oil. It is also a rich source of plant protein. It is advised that for an average Indian adult the dietary protein requirement is 0.8 to 1 g per kg body weight. However, the average intake is about 0.6 g per kg body weight. In India, a large group of population is purely vegetarian; some group of people does not consume even egg and dairy products as well. These people fulfill their

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protein requirement consuming cereals. It was reported that cereal based protein could not compete with proteins that animal products contain (Gulati, 2012). Protein deficiency can cause of long-term damage to our society. Protein deficiency is more dominant in rural areas due to low socio-economic status. Hence, it is necessary to find more options to provide affordable protein rich food source for these group of population especially for poor and malnutrition population. In this view, cottonseed protein can play a great role in view of protein source. However the use of cottonseed for non-ruminants and humans are hampered due to the presence of a toxic compound known as gossypol which denatures the protein when cottonseed is processed (Adams *et al.,* 1960). Gossypol presents in two form free and bound form altogether known as total gossypol. Gossypol toxicity is the major problem in utilization of cottonseed for food purpose and animal feed. In monogastric animals, due to presence of gossypol many problems may occur such as fertility problem, damage of organ, growth disturbance etc. Hence, gossypol reduction is a challenge for the researchers. On the other side, there is a huge production of cottonseed in India which needs to be proper utilized. These cottonseed based products can play a dynamic role to solve malnutrition problem of poor people in India. Cottonseed contains valuable by-products such as 27% hulls, 8% linters, 20% oil and 45 % cottonseed meal (Shaikh *et al.*, 2014). Gossypol reduction to safe level may increase cottonseed's importance in food industry as cottonseed kernels contain 45% crude protein. Numerous studies have been performed for reduction of gossypol such as pressure cooking (Gribbins, 1951), screw pressing (Batson *et al.*, 1951), solvent extraction (Li *et al.*, 2016; Kumar *et al*., 2020), liquid cyclone process (Smith, 1971), microbial fermentation (Shaikh *et al.,* 2014 and Mageshwaran *et al.*, 2015). Although numerous attempts have been made for gossypol reduction however, there is no systematic studies come out so far in India. Also cottonseed based products are still unavailable. Production of low gossypol cottonseed flour requires process optimization. However, the solvent extraction process was observed the most effective method for gossypol reduction (Dechary *et al.*, 1952 and Li *et al.*, 2016). Singh *et al*. (2019) performed study for defatted cottonseed meal using mixed solvents comprises butanol-ethanol-water (80:15:5 v/v) acidified with 0.5 M oxalic acid at solvent to seed ratio 15, extraction time 180 min and temperature 348 K. maximum 94 % gossypol could be extracted using this method. Kuk *et al.* (2005) used mixture of n-hexane and acetone in different concentration for gossypol reduction. They reported that 10:90 (vol/vol) mixture of acetone/hexane reduced free gossypol by 80%. Numerous study were carried out using microbial method. Zhang *et al*. (2018) reported isolation of gossypol degrading bacteria from the cow rumen and its potential for gossypol reduction in cottonseed meal. The study showed that free gossypol reduction by 78.86% was obtained using strain Rumen Bacillus Subtilis (RBS). Shaikh *et al*. (2014) discussed the reduction of gossypol and increase crude protein level in cottonseed cake using mixed culture fermentation during solid state fermentation. It was found that mixed culture fermentation gives better results than single culture fermentation.

In the current research work, three different methods *viz.,* enzymatic method, sprouting method, and solvent method will be applied to study the effect on gossypol reduction, protein rich cottonseed flour production and oil yield from cottonseed. In solvent method four different solvents namely, acetone, ethanol, methanol and iso-propanol will be used with their different levels. In microbial method, cultures of two species, Candida tropical and Saccharomyces cerevisiae and their combinations will be tested.

MATERIAL AND METHODS

Cotton seed hybrid variety (YUVA BG) was obtained from Ginning Training Centre, ICAR-CIRCOT, Nagpur. Cottonseeds were first dehulled at cottonseed oil processing plant, Ginning Training Centre, ICAR-CIRCOT, Nagpur, India. Cottonseed kernels were cleaned manually to remove dust, hulls and other unwanted material from the kernel. Finally cottonseed kernels were ground using electric grinder to make fine powder of 0.8 mm. The kernel powder was used for carrying out experiments. In the present study, three different methods were tested to compare the gossypol reduction in cottonseed.

Enzymatic method :

In this method, microbial treatments were given to the kernel powder. For this purpose, the cultures Candida tropical, Saccharomyces cerevisiae and their combinations were used. The cultures were obtained from Microbiology lab, CIRCOT, Mumbai. For inoculum preparation, 50 ml sterile malt extract (1X) pH 5.5 was

taken into two different 250 ml conical flask. 1% suspension of malt extracts broth cultures were transferred into the flasks individually. The flasks were put on a rotary shaker (150 rpm) for 48 h at 30^0C for incubation. The prepared culture inoculums were used for giving microbial treatments to the cottonseed kernel powder. For treatments, 5 g cottonseed kernel powder was taken into three 100ml conical flasks and then autoclaved at 110^0C for 15 min. After cooling the two flasks were inoculated using respective cultures inoculum. Third flask was inoculated using both the cultures. The flasks were then incubated for 48 h at 30^0C for solid state fermentation. The fermented kernel powder was mixed properly and then tested for free and total gossypol analysis.

Sprouting method:

In this method first, acid delinting was performed on cottonseed to separate short fibres attached on its surface. The cottonseed was washed properly and all the water was drained out. Then they were spread in a plastic tray and a moist cloth was used to cover it. In two days sprouts were come out. The sprouted cottonseed was tested for free and total gossypol determination.

Solvent treatments :

In this method, four different solvents *viz.,* acetone, isopropanol, ethanol and methanol were used at different levels to treat the cottonseed kernel powder for free and total gossypol reduction. All the treatments to reduce free and total gossypol were provided before oil extraction. For experiment, 5 g kernel powder was taken into 100 ml conical flask for each experiment. The water mixed solvent was used into the conical flask and put into a rotary shaker for 60 min at 100 rpm. After that it was filtered using a filter paper (whatman no. 4). The solid residue was dried and used for free and total gossypol determination. The procedure followed has shown in Fig A. In this method solid to liquid ration was fixed to 1:5. Each solvent was used at four different levels *i.e.*70%, 80%, 90 % and 100% as given in Table 2.

Analytical method :

The free and total gossypol was determined by American Oil Chemists Society (AOCS, 1986) official methods Ba 7-58 and American Oil Chemists Society (AOCS, 1986) official methods Ba 8-78, respectively.

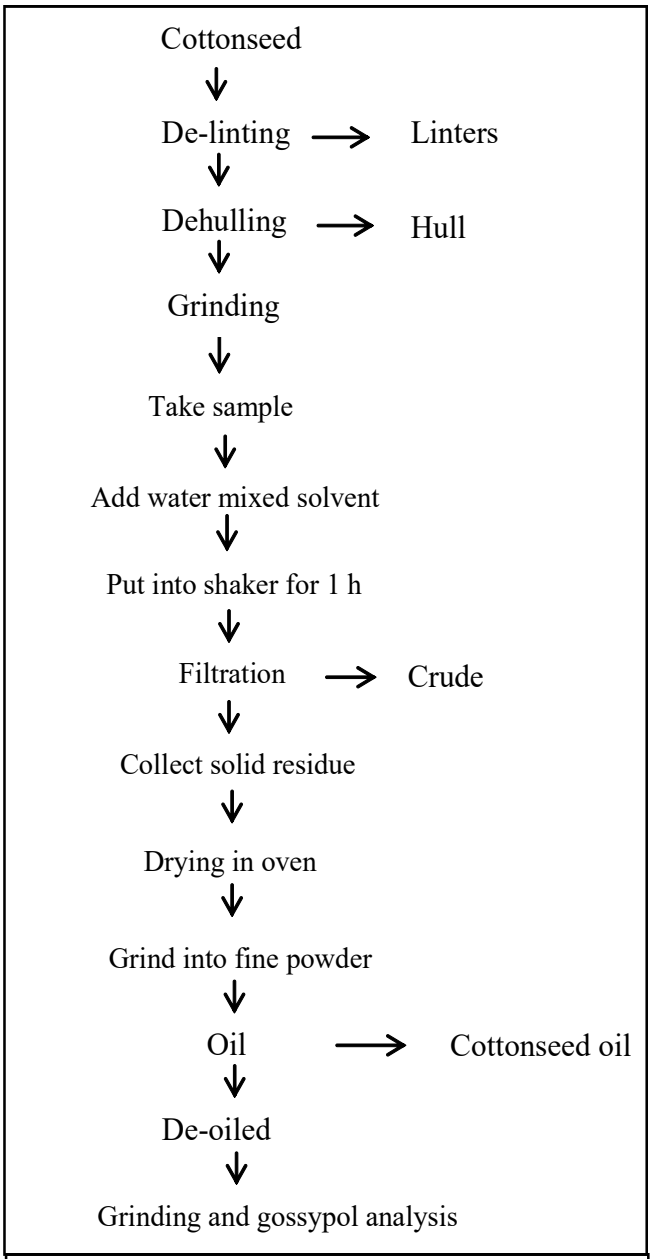

Fig. 1: Gossypol extraction using solvent method

Moisture content was determined by drying the sample in a convection hot air oven at 105^0C for 5hrs. The crude protein content was determined using Kjeldahl's method. The oil content was determined by solvent extraction method using n-hexane. The yield of final product was determined by knowing the difference of initial and final weight of the sample.

RESULTS AND DISCUSSION

The present study was performed to compare the

effect of various treatments *viz.,* microbial treatments, sprouting treatment and chemical treatments on free gossypol %, total gossypol %, oil content, flour yield and crude protein content of cottonseed flour. The results of all the three types of methods have shown in Table 2. Among all methods the lowest value of free gossypol content (0.03%) was obtained using 70 % aqueous acetone however there was not much reduction in total gossypol content which was found as 1.47% also the crude protein content in flour was obtained highest 65.6 % using 70% aqueous acetone. Among all method, the lowest value of total gossypol (0.83%) was recorded in enzymatic method using culture candida tropical (CT). The free gossypol content (0.08%) was also observed less in this treatment while 39.4 % crude protein was achieved in the final flour. Among all the three methods, solvent method showed better results in respect of free gossypol reduction, crude protein content, oil content and flour yield. In solvent method, four solvents and their different levels have shown in Table 2.

In the present study, sprouted cottonseed did not showed any significant effect on gossypol reduction and protein content improvement. In enzymatic method, culture of candida tropical, (CT) performed better in respect of free and total gossypol reduction. However, there was not much improvement in crude protein content. Similar findings were also found by Shaikh *et al*. (2014) where defatted cottonseed meal was tasted.

The influence of different solvents on free gossypol content has shown in Fig. 1. It can be seen that 70% aqueous acetone showed lowest free gossypol content that is desirable. As acetone percentage is increasing the free gossypol content in cottonseed is also increasing. The result was similar to the study carried out by Pelitire

Table 1: Initial value cottonseed meal

Treatments	Cottonseed powder (g)	FG%	TG (%)	Oil %	Protein%
Cottonseed defatted meal (without treatment)	5	1.06	3.05	21%	30

Table 2 : Effect of various treatments on FG, TG, oil per cent, Flour yield and protein content

Sr. No.	Treatments	FG (%)	TG (%)	Oil (%)	Flour yield (%)	Protein (%)
1.	**Microbial treatments**					
	Candida tropical, (CT)	0.08	0.83	31.4	62.4	39.4
	Saccharomyces cerevisiae (SC)	0.071	1.18	30.6	61	35
	CT+ SC	0.073	1.06	31.1	63.8	36
2.	**Sprouting treatments**					
	Sprouted cottonseed	1.15	2.9	32	66	35
3.	**Chemical treatments**					
	70 % Acetone	0.03	1.47	31.3	57.2	65.6
	80 % Acetone	0.066	1.4	32.7	57.8	55
	90 % Acetone	0.09	1.32	33.6	58.9	54
	100% Acetone	0.19	1.9	18.24	60	50
	70 % Ethanol	0.11	1.65	31.8	56.1	52
	80 % Ethanol	0.18	1.85	32.8	56.7	52.5
	90 % Ethanol	0.26	1.93	34.5	57.6	53
	100% Ethanol	0.5	2.32	24.97	59.4	52
	70 % Iso-propanol	0.13	1.7	31.4	57.2	52.7
	80 % Iso-propanol	0.17	1.91	30.76	58	52
	90 % Iso-propanol	0.34	2.09	25.54	62.4	51
	100% Iso-propanol	0.6	2.7	13.1	62.8	49
	70 % Methanol	0.219	2.92	39.08	52	53
	80 % Methanol	0.174	2.56	39.64	53.3	52.7
	90 % Methanol	0.159	2.17	33.49	58	51.5
	100% Methanol	0.09	1.87	25.2	63.8	50

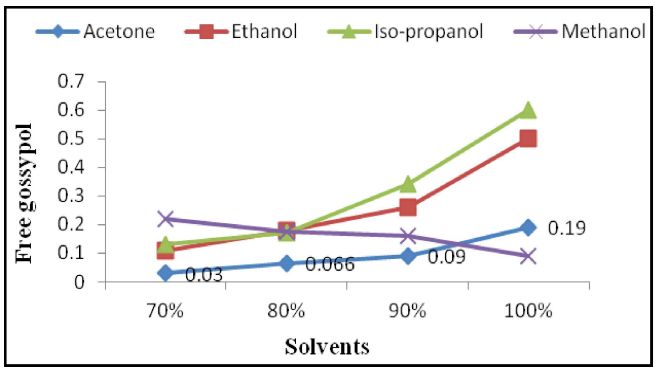

Fig. 1 : Effect of various solvents on free gossypol percentage

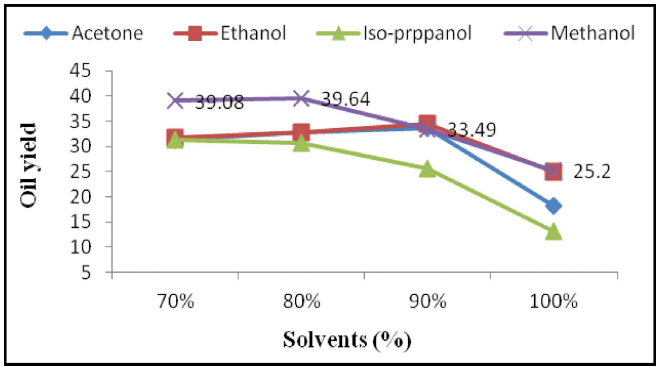

Fig. 3 : Effect of different solvents on oil yield (%)

et al., 2014. Iso-propanol and ethanol both are showing the same trends as well. While in case of methanol reverse trend was obtained, where maximum percentage of free gossypol was observed at 70% level while minimum free gossypol content was found at 100% methanol level.

The effects of various solvents on total gossypol content have shown in Fig. 2. Among all the solvents the lowest value of total gossypol was obtained using 90% aqueous acetone as shown in Fig. 2. In case of other solvents such as ethanol and iso-propanol showed their lower value of total gossypol at lower level of solvents while higher value of total gossypol obtained at higher level of solvent as shown in Fig. 2. However, in case of methanol, total gossypol was at their lowest level using 100% methanol.

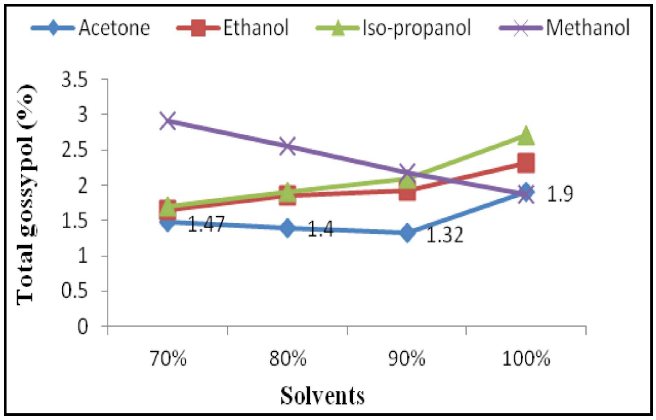

Fig. 2 : Effect of various solvents on total gossypol percentage

Solvent treated cottonseed powder was defatted for oil recovery. In this reference, effect of various solvents on oil yield was analyzed. Maximum oil recovery in cottonseed powder was recorded as 39.64 % using 80% methanol as shown in Fig. 3. However, free and total gossypol content at 80% methanol level was much higher (0.174 and 2.56, respectively) that is undesirable.

Flour yield of solvent treated followed by defatted meal was determined. From Fig. 4 it can be seen that all the solvents had given maximum flour yield at their higher level *i.e.* 100% level. However, at higher level of solvents there was not much reduction in free and total gossypol. Since acetone had shown better results relating to free gossypol and total gossypol reduction, oil content etc. therefore flour yield was recorded for all levels of acetone as shown in Fig. 4. It can be seen that at lower level of acetone (70%) flour yield was 57.2%. Flour yield increases with increase in acetone level.

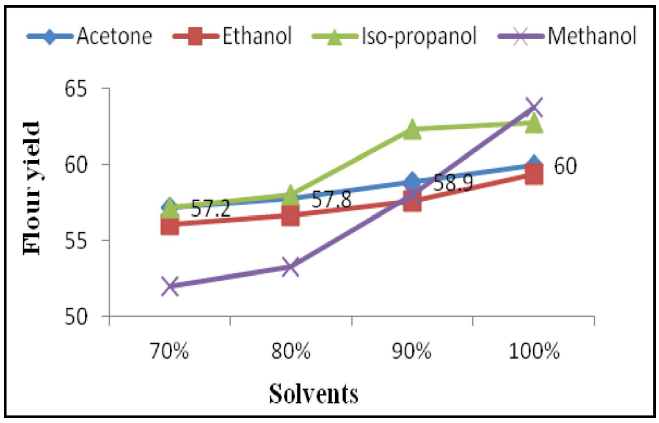

Fig. 4 : Effect of different solvents on flour yield (%)

In the present study, crude protein content of defatted cottonseed flour was also determined. Sample treated with 70% acetone shown maximum crude protein (65.6 %) in the cottonseed flour. Further increase in acetone level decreased crude protein percentage. Other solvents treated meal shown lower crude protein content compare to acetone as shown in Fig. 5.

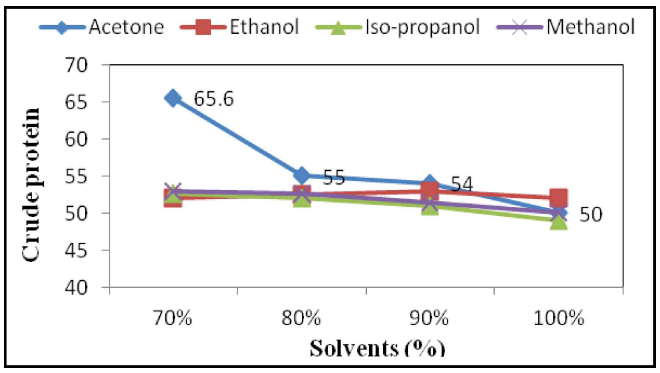

Fig. 5 : Effect of different solvents on crude protein content of cotton seed flour

Conclusion :

The present study was carried out to compare three different methods *viz.,* sprouting method, enzymatic method and solvent method in view of gossypol reduction, crude protein content and oil content in cottonseed, and flour yield. Research revealed that solvent treatment is better than enzymatic treatment in view of free gossypol reduction and crude protein content in flour. The results showed that 70% aqueous acetone performed better in respect of free gossypol reduction (97%), crude protein content (65.6%) and flour yield (57.2%) among all treatments.

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