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COMPARATIVE EXTRACTION OF COTTONSEED OIL BY n-Hexane and Ethanol

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ABSTRACT

Cottonseed is a rich source of oil and protein. Cottonseed oil has gained importance as heart healthy oil as it contains lots of un-saturates. It is accepted as a better medium for frying foods. Presently, n-hexane is the solvent of choice in most of the solvent extraction plants though it has been graded as highly toxic and hazardous for the environment. The present study is undertaken to explore the possibility of using ethanol as a non toxic and green solvent in place of n-hexane. The extraction of cottonseed at different temperatures, solvent-solid ratio and particle size at different time intervals using the two solvents is presented to compare the extraction efficiency of the two solvents. The results indicate that at temperature of 45^{o}C and solvent to solid ratio of more than 10, ethanol has oil extraction efficiency comparable to hexane. The meal produced in the extraction process is found to have lower gossypol content when ethanol is used.

Keywords: cottonseed oil, solvent extraction, n-hexane, ethanol, G. Hirsutum, comparability.

INTRODUCTION

Cotton is a cash crop for more than 20 million farmers in developing countries of Asia and Africa. It is mainly cultivated to meet the basic requirement for cotton fabrics. Cottonseed is a valuable by-product of the cotton plant and for every kg of cotton fiber, 1.65 kg of cottonseed is produced (Rathore, 2007). Global cotton cultivation in 2009-10 yielded 23.3 million metric tons (MMT) of cotton and around 40 MMT of cottonseed (Cotton Incorporated, 2010, Aug. 13). China tops in production of cotton in the world, whereas India stands second (Shekhar, 2006). Cottonseed contains approximately 18-25% of oil and 20-25 % high quality protein. The global annual production of cotton seed could potentially meet the total protein requirement of nearly half a billion people for a year @50 grams / day (Rathore, 2007) but presently cottonseed is not used in food preparations. It is used in animal feed in regulated manner due to the presence of gossypol. Cottonseed oil is rich in tocopherols which inhibits rancidity development and thus contribute to its stability resulting in a longer shelf life for the product. Cottonseed oil is naturally hydrogenated oil and is suitable for heart due to the presence of palmtic, stearic, mysteric, oleic, linoleic and linoleinic fatty acids in sufficient quantities. Cottonseed oil has also gained importance in food preparations due to its higher smoke point (about 232^{o}C) compared to other cooking oils and is good for frying food articles (Brien *et al*., 2005). Refined Cottonseed oil has a mild taste and light golden color. It also finds a number of other non food uses in biodiesel production, in paint industry and as an environmentally accepted lubricant additive to improve the lubricating abilities of the base oil SAE 20 W50 (Ertugrul *et al*., 2004).

Solvent extraction is the commonly used commercial technique to recover oil from oilseeds, Presently n-hexane is the preferred solvent throughout the world due to its extraction efficiency and ease of availability yet hexane has been categorized as a hazardous air pollutant (HAP) by the US Environmental Protection Agency and is included in the list of toxic chemicals (NIOSH. 2007). The maximum permissible limit for n-hexane in oil and the meal are 5ppm and 10ppm, respectively (PFA act 1954). It is very tedious and energy consuming to reduce hexane concentration in the meal up to or below maximum permissible limit. These problems have attracted researchers to find a suitable alternative solvent. A number of solvents and their mixtures like heptane, acetone, ethanol-water azeotrope, methyl-pentane, isohexane, petroleum-ether, trichloroethane, chlorinated hydrocarbons, alcohols etc. for oil extraction from oilseeds have been reported in literature (Ayers *et al*., 1951; Conkerton *et al*., 1995; Wan *et al*., 1995(a) and (b); Gandhi *et al*., 2003; Maria *et al*., 2008; Frampton *et al*., 1967; Kuk. *et al*., 2005; Kuk, 1998; Sepidar *et al*., 2009; Johnson *et al*., 1983). Out of these, use of alcohols is gaining attention due to their higher threshold limit in the environment. Junfung (2010) has reported simultaneous extraction of oil and gossypol using mixture of methanol and hexane thereby lowering gossypol content to 0.014% thereby making the cottonseed cake suitable for feed. Although very good results have been reported by him for extraction of oil as well as gossypol still the hexane content in the cake is not within the acceptable limits. Bhowmick (2003) have advocated the use of isopropanol due to the higher solvency of oil and gossypol in it. In the alcohol series, ethanol is the safest solvent as it is obtained from biological sources by the fermentation process and is placed in the category of GRAS (generally recognized as safe). Very few studies are reported for the use of aqueous ethanol as solvent. Hron *et al*., (1994) have reported a two step process using 95% ethanol for extraction of oil from cottonseed. The extraction process consists of extraction at lower and higher temperatures; the lower temperature facilitates the reduction of gossypol concentration in the meal while higher temperature facilitates oil extraction. Further, it is reported that at higher temperature the extraction of

gossypol was not possible as it combines with protein present in cottonseed. No detailed data are available in the literature regarding oil extraction from cottonseed with absolute ethanol. Therefore, the study has been conducted to compare the efficiency of oil extraction from cottonseed by n-hexane and ethanol at temperature less than 45°C to explore the possibility of using ethanol as a solvent to obtain oil as well as good quality of cake suitable for food/ feed containing lower gossypol content. This paper discusses the effect of parameters viz. temperature, particle size (PS), solvent-solid ratio (SR), extraction time on the oil extraction efficiency.

MATERIALS AND METHODS

Cottonseed

Acid delinted cottonseed of *G. Hirsutum* (P8-6) was obtained from IARI, Pusa, N. Delhi. The seeds were de-hulled manually using a mixer grinder. The de-hulled seeds were dried at 45°C in vacuum oven followed by segregation in to three fractions A, B, and C using standard sieves as shown in Table-1. The seeds were analyzed for oil content by an exhaustive extraction method using hexane in a soxhlet apparatus. The de-hulled seeds were found to contain 36.87% oil on dry solid basis.

Table-1. Average particle size of cottonseed meal.

S. No.	Particle grade	Mesh size	Average particle size (mm)
1	A	-14	0.6
2	B	-8+14	1.6
3	C	-6+8	2.4

Solvents

The Laboratory Reagent Grade solvents n-Hexane and Ethanol were procured from Fisher and were redistilled before oil extraction.

Oil analysis

a) Solid sample

The oil content in solid seed samples were analyzed by an exhaustive extraction method using hexane in a soxhlet apparatus as per method described in Appendix F of IS: 7847-1968 of Bureau of Indian Standards [IS: 7847-1968].

b) Liquid sample

The determination of oil in liquid samples was carried out by measuring the density of the extracted solvent. The change in the density of the solvent is directly proportional to the amount of oil dissolved in the solvent. Mettler Toledo make density meter, Model DE-45 Delta Range was used to record the change in density up to 4 decimal places. Firstly, the calibration curves were prepared using raw cottonseed oil extracted by n-hexane and ethanol respectively. Subsequently, a known quantity of oil was dissolved in the same solvent at different dilutions, the density of each dilution was measured and calibration curve, density v/s oil concentration (gm oil/L solvent), was prepared. To determine oil content in liquid phase, the density of the clear liquid portion separated after centrifugation was obtained. The oil content in the sample was calculated using the calibration curve. For few representative clear liquid samples, the results were cross-checked for oil content by evaporating solvent in vacuum oven to obtain solvent-free oil (Conkerton *et al*., 1995).

Gossypol analysis

c) Gossypol was tested in the seed by the method described in IS: 7847-1968 of Bureau of Indian Standards. In this method total gossypol is completely removed from meal in a 30 minute extraction during which gossypol is complexed with neutralized 3-amino-1-propanol in N-N dimethyl formamide. The difference in absorption of aliquot portion of the extract before and after reaction with aniline serves as a measure of total gossypol content. A calibration curve is prepared making solutions of different concentration using standard gossypol (Sigma Aldrich make) and absorbance at 440 mμ is recorded using UV-Vis double beam spectrophotometer (EC Make) and the calibration curve is plotted between gossypol concentration (mg/ml) v/s absorbance. Gossypol is determined in the sample aliquot by means of calibration curve as mg. Total Gossypol is determined using formula below.

$$\text{Total Gossypol}\,(\%) = \frac{5 \text{ X } M_1}{M_2 \text{X } V} \qquad \text{------(1)}$$

Where

M_1 = Weight of gossypol in sample aliquot in mg
M_2 =Weight of sample used for analysis in g
V =Volume of aliquot taken in ml

Oil extraction

The required quantity of cottonseed samples was weighed in airtight 30 ml plastic bottles. These bottles along with the solvent bottle were kept overnight in constant temperature incubator to attain desired uniform temperature. The measured quantity of solvent was then taken out and mixed with the seed sample kept in the bottles for extraction of oil. After thorough mixing, the bottles were closed airtight and kept immediately in the shaking incubator. The study was conducted for a period of three hours and one plastic bottle was taken out after specified period of 1, 5, 10, 20, 40, 60, 120 and 180 minutes, the supernatant liquid was taken out from the plastic bottle and centrifuged at 9000 rpm for 5 minutes. The clear transparent liquid obtained after centrifuge was taken out for oil determination. The studies for extraction were carried out at four temperatures i.e., 15, 25, 35, and 45$\pm$1°C, three solvent-seed ratios (5, 10 and 15) and three particle sizes i.e. A, B and C. Percentage of oil extracted was calculated using Equation (2). Experiments were run in duplicate and the average value was taken to ensure

accuracy and the results were expressed on dry sample basis.

$$Percent\ Oil\ Extracted(\%) = \frac{C_t \text{ X } S}{w_o} X100 \qquad \text{------(2)}$$

Where: C_t=Oil concentration in solvent at any time, t (g/L)

S = Volume of solvent mixed with seed sample (L)
w_o= Weight of total oil present in the seed sample (g)

RESULTS AND DISCUSSIONS

Effect of extraction time

The extraction time is an important parameter for solvent extraction. It helps in deciding the optimum residence time required in extractor. Thus extraction of oil from the cottonseeds of particle size (PS "A" was carried out at 35°C and SR of 5 with both the solvents. Extraction data obtained from PS "A" up to 180 minutes are plotted in Figure-1. The results show that extraction of oil increased with time for both the solvents. It is also observed that the rate of extraction is very high during the first 20 minutes of extraction and afterwards it tapers off. The maximum oil recovered is 57.7% and 74.14% with ethanol and hexane respectively when the extraction process was carried out for 180 minutes. About 22-33% oil was extracted instantaneously and approximately 90-95% of the total oil was extracted in about 180 minutes. The extraction data was, therefore, obtained for 180 minutes. The initial high rate of extraction may be due to quick solubility of the oil present at the solid surface and higher mass transfer driving force provided by low oil concentration in the fresh solvent. The later slower rate may be attributed to lower driving force due to increasing oil concentration in the solvent (Treybal,1968).

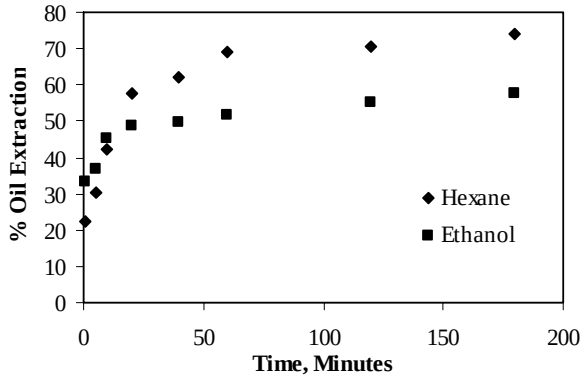

Figure 1: Effect of Extraction Time on Oil Extraction at 35 °C, PS "A" and SR:5

Effect of solvent to solid ratio (SR)

The ratio of solvent to solid is another important parameter for extraction process. In the present study experiments were conducted with solvent to solid ratios of 5, 10 and 15. The data are plotted in Figure-2 and Figure-3 for oil extraction using hexane and ethanol respectively at 35°C from particle size-A. Figures show that oil extraction increases with increase in SR from 5 to 15 for both solvents. In case of ethanol the maximum extraction was found to be 65.17, 81.37 and 86.43% at SR of 5, 10, and 15, respectively while in the case of hexane it was 74.14, 85.25 and 88.72% at SR of 5, 10, and 15, respectively. At lower SR the oil extraction may be restricted due to the lower solubility limit of oil in solvent. Further it can be seen that percentage increase in oil extraction was low when SR was increased from 10 to 15 as compared to increase in oil extraction when SR was increased from 5 to 10. Hence further study was restricted to solvent-solid ratio of 10 and 15.

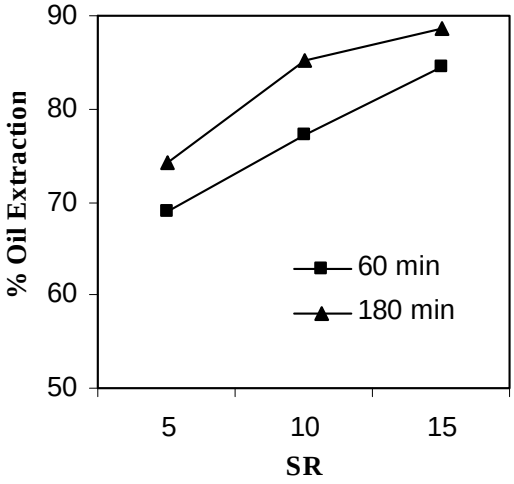

Figure 2 : Effect of SR on Oil Extraction by Hexane at 35 °C PS "A"

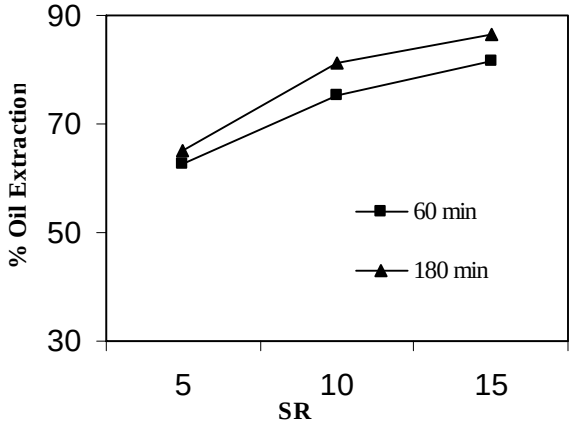

Figure 3: Effect of SR on Oil Extraction by Ethanol at 35 °C, PS "A"

Effect of particle size

The extraction was carried out from the seeds of 3 sizes as mentioned in Table-1 by both the solvents at 35°C with the SR of 10. The results are shown in Figure-4 and Figure-5. It may be seen from these Figures that initial slopes of extraction curve are lower for particle size "B" and "C" as compared to slope for extraction curve with particle size "A" indicating slow extraction rate from coarser particles compared to fine particles. Further it

could be seen that the maximum oil extraction using hexane is (85.25%) from PS "A" compared to (49.37%) from coarser particle size "B". Similar trend is also found with ethanol. Thus percentage extraction with fine particle is nearly double with that of coarse particle "B" by both solvents. The lower extraction rate can be attributed to the fact that the bigger particles have less surface area directly exposed to the solvent as compared to the smaller size and the solvent has to penetrate into the core of the seed to leach the oil out of the seed which restricts the rate of oil extraction from the bigger seed particles.

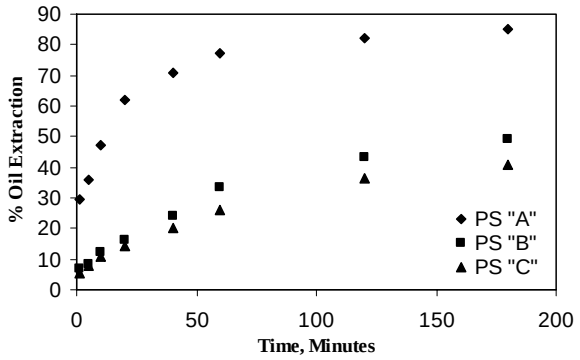

Figure 4: Effect of Particle Size on Oil Extraction by Hexane at 35 °C, SR : 10

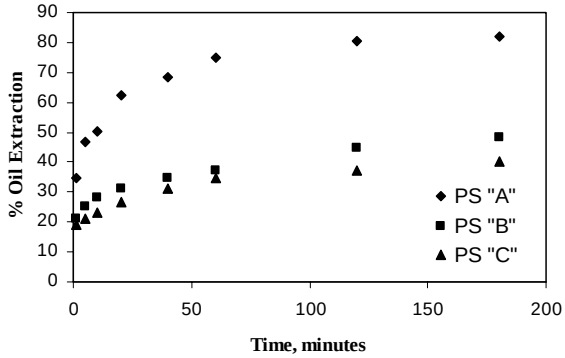

Figure 5: Effect of Particle Size on Oil Extraction by Ethanol at 35 °C, SR : 10

Effect of extraction temperature

Temperature is the most important parameter for solvent extraction specifically in case of oil extraction from cottonseed as it affects the quality of oil. Cottonseed contains gossypol, a polyphenolic compound, which is toxic in nature for living beings. At higher temperature of about 70°C it reacts with lysine, which is one of the important essential amino-acid present in cottonseed resulting in reduced availability of protein in the cottonseed meal. Hence studies were conducted at lower temperature to prevent any reaction between gossypol and lysine. In the present work, the experiments were conducted at four temperatures 15, 25, 35 and 45°C. The data was plotted in Figures 6 to 9. It was found that increase in extraction temperature has a direct effect on the amount of oil extracted.

It may be seen that the amount of extracted oil with hexane in 180 minutes increased from 40.07 to 92.84 % (Figure-6) and 70.49 to 91.14 (Figure-8) by increasing the extraction temperature from 15°C to 45°C. Whereas while using the ethanol as solvent the increase in extraction was observed to 48.07-93.57% (Figure-7) and 62.86-94.8 (Figure-9) in increasing the temperature from 15°C to 45°C. It may be explained on the fact that at higher temp (45°C) the solubility of ethanol improved.

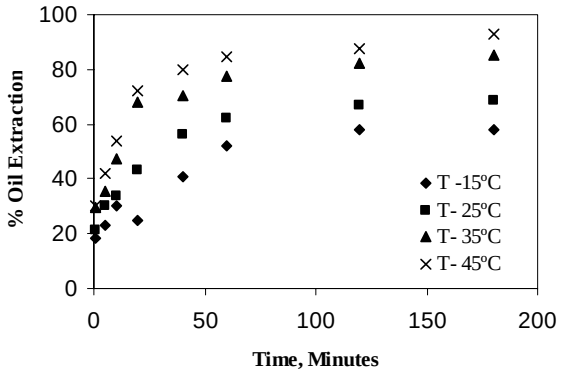

Figure 6: Effect of temp.on Oil Extraction by Hexane at SR : 10, PS 'A'

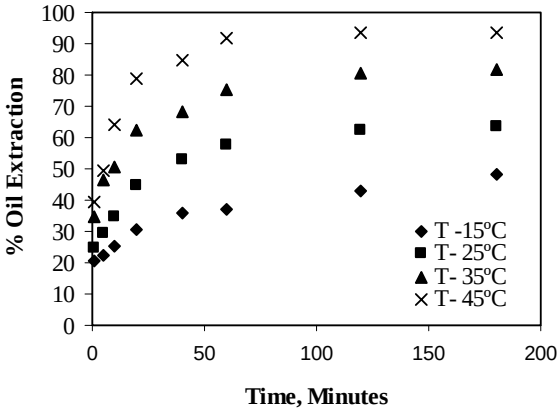

Figure 7: Effect of temp. on Oil extraction by Ethanol at SR: 10, PS "A"

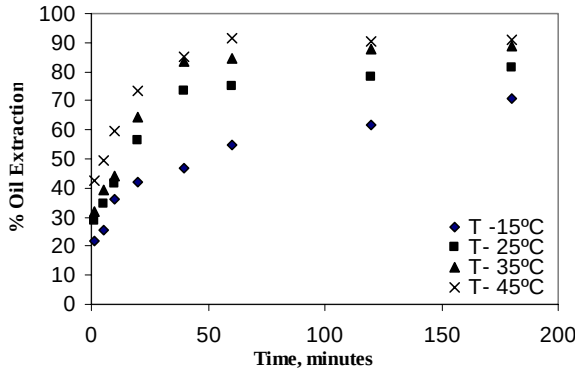

Figure 8: Effect of Temp. on Oil Extraction by Hexane at SR: 15, PS"A"

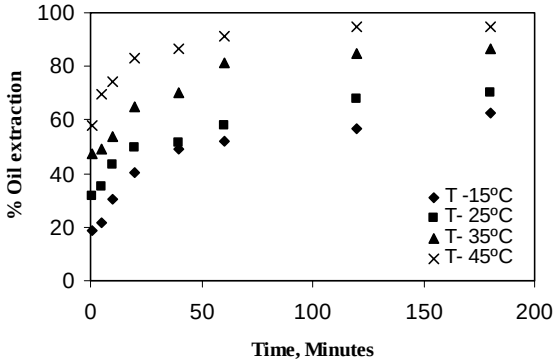

Figure 9: Effect of temp on Oil Extraction by Ethanol at SR :15, PS "A"

Effect of solvent on extraction

Cottonseed meal of PS "A" was extracted seven times using both the solvents Hexane and Ethanol at SR 10 at 35 o C. After each extraction for one hour, the cottonseed meal was separated, dried, weighed and analyzed for gossypol and oil content. After first extraction the seeds were re extracted using the same solvent and solvent ratio. The data was plotted in Figures 10 and 11. The result indicates (Figure-10) that maximum oil was extracted in first extraction i.e., 77.27% and 75.15% by hexane and ethanol respectively showing a reduction of approx 2.12% in extraction efficiency of ethanol as compared to hexane. The oil extraction rate for both the solvents was reduced in subsequent extractions, after 4th extraction, the total extraction of oil by hexane and ethanol was 99.08 and 97.84 % leaving a difference of 1.24% less extraction by ethanol. Thereafter the rate of extraction was too low and at the end of 7th extraction, more than 99% oil was extracted by both solvents (99.75 and 99.11% oil extraction by hexane and ethanol).

At low SR ratio the extraction of oil from ethanol was found to be low as compared to the oil extracted by hexane under similar conditions. At higher solvent ratio the efficiency of oil extraction with ethanol was found to be comparable with hexane at 45^{o} C at SR of 10 with particle size "A". 97.8% with hexane and 96.2% by ethanol. The low extraction of oil with ethanol at low solvent ratio can be attributed to the solubility of cottonseed oil in ethanol. The oil extracted by n-hexane and ethanol was tested for some parameters and it is observed that there is no much variation in the quality of the oil extracted by hexane and ethanol except that the color of the oil extracted by ethanol is darker than the oil extracted by hexane which indicates that, ethanol is a good solvent for gossypol pigment of the seed and is extracted by ethanol along with the oil. Acid value which is the indicator of free fatty acid present in the oil is slightly high in the oil extracted by ethanol. Major variations were not observed in other parameters of oil like Iodine value, Saponification value etc. Proper refining of the ethanol extracted oil will reduce the gossypol within the limits in making it fit for human consumption.

The comparative extraction of gossypol using both the solvents is shown in Figure-11. Which indicates that about 70% gossypol can be recovered by ethanol as compared to about 20% extraction of gossypol by hexane under similar conditions making the cottonseed meal more suitable for feed/ food uses.

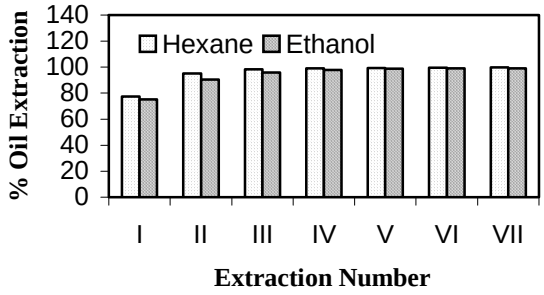

Figure 10:Comparative Extraction of Oil

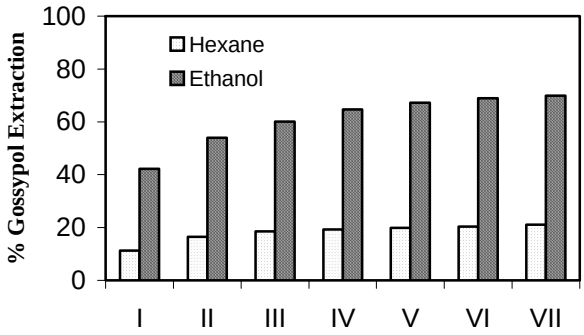

Figure 11: Comparative Extraction of gossypol

CONCLUSIONS

Based on the observations made above, it can be concluded that ethanol, a green and safe solvent can be a better alternative to n-hexane. The extraction efficiencies of both the solvents are comparable at 45^{o}C at a SR of 10:1 and higher. Lower particle size has better extraction efficiency. The cottonseed meal left after extraction contains about 50% less gossypol content is case of ethanol compared to hexane. More cottonseed meal can thus be utilized in feed than the present practice. A detailed techno-economic feasibility study of process based on ethanol is underway.

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