

# Kinetics and Thermodynamics of Gossypol Extraction from Defatted Cottonseed Meal by Ethanol Acidified by Oxalic Acid

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**Abstract:** Cotton crop is grown to meet the basic requirement of fabrics. After de-linting the cotton ball, seed is left which is a good source of oil approx.18% and protein approx.25%. After extraction of oil from the seed, the left over cake is a rich source of good quality protein but presence of gossypol, a highly toxic substance, makes it unfit for human consumption. A number of earlier attempts to extract gossypol from the seeds using different solvents have been made to reduce the gossypol content within the prescribed maximum FDA limit of 450 ppm. In most of the cases, gossypol extraction had been carried out at low temperatures to minimize its binding with lysine (an essential amino acid present in the protein of the seed), as higher temperature accelerated the binding process which resulted in reduced digestibility of the protein. Ethanol acidified with mild organic acids has been reported to prevent complexing of gossypol with lysine at relatively higher temperatures. Present studies are undertaken to extract gossypol from the defatted cotton seed meal using green solvent (ethanol) acidified by mild organic oxalic acid at temperatures up to 343K to improve extraction efficiency. Up to 92.4% gossypol could be extracted from the defatted cottonseed meal using acidic ethanol as against 61.55% extraction with pure ethanol thus making the seed meal suitable for food uses. The data obtained were analyzed to establish kinetics and thermodynamics of the extraction process which would be helpful in scaling up the process to pilot/ commercial scale.

**Keywords:** Cottonseed Meal, Gossypol, Acidic Ethanol, Toxicity, Food Uses

## 1. Introduction

Cottonseed, a bye product of cotton harvesting is a rich source of oil and protein. After oil extraction, the defatted cottonseed meal contains more than 40% good quality protein that can meet the protein requirement of about 50 million people annually [1]. Gossypol, a toxic substance, is naturally present in all parts of the cotton plant in the form of tiny size glands to protect the plant from insects, pests and diseases. Its presence make the seed protein unfit for human consumption. Gossypol comes out of the ruptured glands during processing and complexes with lysine present in the cottonseed protein to form bound gossypol. Though the bound gossypol is not toxic still during the complicated digestion process it may become a safety concern. Thus separation of gossypol from the seed is important not only for better utilization of cottonseed meal but also gossypol can be suitably used. Gossypol is a valuable substance in spite of its toxicity and has gained importance due to its diversified uses viz fabric dyes [2], antioxidants, stabilizer for vitamin A [3], antioxidant-stabilizer for PVC/ Polypropylene, in cement mixtures to increases the life of road and cold resistance and decreases water absorption, antioxidant in the petroleum and rubber industry [4],[5], male contraceptive, to prevent hemorrhaging for stimulating menstrual flow and make child birth easier, as antimicrobial and effective in wound healing [6], for treatment of scalp infection, dysentery, gonorrhoea and to inhibit replication of the HIV-1 virus [wikipedia], anticancer drug [7], for control of urban and agricultural pests etc.

Among the different techniques to separate gossypol solvent extraction remains the most popular and has been tried by most of the researchers. The choice of solvent is based on solubility of gossypol in the selected solvent, cost and safety. Considerable work has been reported using solvents like ethyl ether [8], hexane [9], [10], acetone [11], methyl ethyl ketone-phosphoric acid mixture, isopropanol [12], acetone/hexane mixture [13], isopropyl alcohol hexane mixture[10], water mixture with butanone, trichloroethylene, perchloro ethylene [14] for separation of gossypol from cottonseed. It has been reported [15] that simultaneous extraction of oil and gossypol using mixture of methanol hexane mixture. Although good results for extraction of oil as well as gossypol have been reported but solvent content in the cake remains a big concern. Thus, there is a need to develop a technique either to produce the seed meal with acceptable limit of solvent or use a solvent that is acceptable in the feed up to a higher limit.

Use of alcohols is gaining attention due to their higher threshold limit in the environment [12]. Out of various solvents, ethanol is found to be the safest solvent in alcohol series that can be used in cottonseed meal processing [16], Hron et. al. 1992, [17] have reported that the complexing of gossypol could be controlled using acidic solvent even at higher temperatures. Keeping this in mind acidified ethanol has been used for extraction of gossypol from cottonseed at temperatures up to 343K in this study to explore the possibility of improving extraction efficiency of gossypol so that good quality cottonseed meal suitable for food/ feed may be obtained in a cost effective manner. The effect of important parameters such as temperature, SR, and extraction time on extraction efficiency has been studied.

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The data obtained are used to discuss the kinetics and thermodynamics of the extraction process.

## 2. Materials and Methods

**Solvents:** Laboratory Reagent grade n-hexane, ethanol and oxalic acid supplied by Fisher India were used to de-fat seeds and extract gossypol. Hexane and ethanol were redistilled before the actual use.

### 2.1 Cotton Seeds

Cottonseed (variety P8-6) obtained from Indian Agricultural Research Institute, Pusa, New Delhi were dehulled in a mixer grinder. The dehulled seeds were analyzed to have fat content of 36.875% on moisture free basis. The dehulled seeds were dried at 318K in vacuum oven. The dried seeds were again processed in the mixer grinder followed by segregation using standard sieves to get average particle size of 0.60mm.

### 2.2 Oil Extraction

50gm of these cottonseeds and 500ml n-hexane were placed in sealed air tight container and kept in the shaking incubator maintained at 308K for 60minutes to extract oil. The supernatant liquid was separated from the solid seeds and tested for oil content as per method described in literature [16]. The procedure was repeated for maximum oil removal. The total extraction of oil by the end of 7<sup>th</sup> extraction was 99.7%. After oil extraction the seeds were kept overnight in the vacuum oven at 308K, 700mm Hg vacuum for removal of n-hexane. Gossypol in the defatted seeds (DFS) was found to be 1.95%.

### 2.3 Gossypol Analysis

Total gossypol content in the seed was determined using ECIL make double beam UV-Visible spectrophotometer, Model: UV5704SS, as per standard method given in Bureau of Indian Standards (IS: 4874-1968). The method involves the development of colored complex of aniline with gossypol extracted with neutralized 3-amino-1-propanol in dimethyl formamide and measuring absorbance at 440nm. Standard solution was prepared using gossypol standard obtained from Sigma Aldrich.

### 2.4 Study

Weighted amount of DFS samples were taken in six 30 ml air tight plastic bottles and kept at required temperature. A measured quantity of alcohol at required temperature was poured in the bottles containing DFS and bottles were closed and kept immediately in shaking incubator. The study was conducted for a period of three hours and the six bottles were drawn one by one after a period of 5,10,30,60,120 and 180 minutes respectively. The seeds were separated by filtration under vacuum, dried and tested for residual gossypol. Gossypol concentration in the liquid was determined by mass balance using the following equation:

$$C = \frac{(C_o - C_s)F}{V} \quad \text{---(1)}$$

Where  $C$  = Gossypol concentration in solvent at any time,  $t$  (mg/ml)

$C_o$  = Gossypol content in DFS initially (mg/mg)

$C_s$  = Gossypol content in DFS at any time  $t$  (mg/mg)

$F$  = Wt of DFS sample taken for extraction (mg)

$V$  = Volume of solvent used for extraction (ml)

## 3. Results and Discussion

### 3.1 Effect of Acid Concentration

To study the effect of acid concentration on gossypol extraction, ethanol was acidified with 0.1M to 0.6M acid solution in ethanol was used for extraction of gossypol from DFS meal at a temperature of 308 K at SR: 10. Figure 1 shows that the percentage gossypol extraction increased with increase in concentration of the acid used for acidifying ethanol up to 0.5M but after this level no appreciable increase in extraction was noticed. Accordingly 0.5M acid concentration was used during further trials

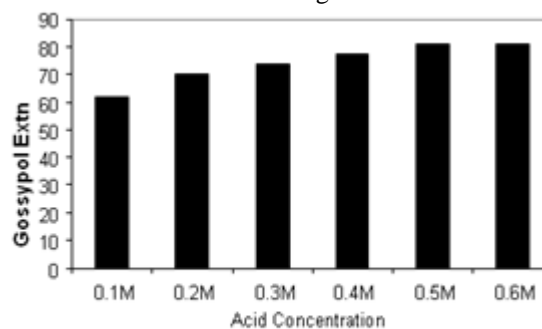


Figure 1: Effect of acid concentration on gossypol extraction by acidic ethanol at 328K

### 3.2 Effect of Solvent to Solid Ratio

The effect of SR on percentage gossypol extraction with ethanol acidified by 0.5 M acid from defatted cottonseed meal at temperature 343 K was studied. The results are shown in Figure 2 for solvent ratio of 5, 10, 15, 20 and 25. The extraction at SR: 5 was found to increase with increase in solid to solvent ratio from 5 (84%) to 15(92.4%) after that no further increase in extraction was observed by increase in SR to 20 and 25. On the basis of the above findings, further studies on SR were carried out ranging from 5 to 15.

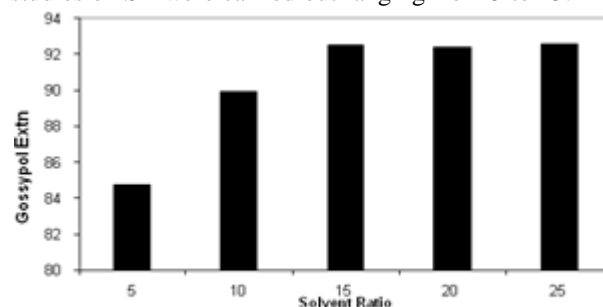


Figure 2: Effect of gossypol extraction on SR by acidic ethanol at 323K

### 3.3 Effect of Time

Extraction of gossypol from DFS meal using 0.5M oxalic acid in ethanol was carried out at 308 K. The results shown in Figure 3 indicate that the gossypol extraction increases initially and most of gossypol is extracted within 2 hours afterwards the extraction is very slow. Similar extraction results were obtained at SR: 10 and SR:15, therefore further experiments were carried out up to 3 hrs only .

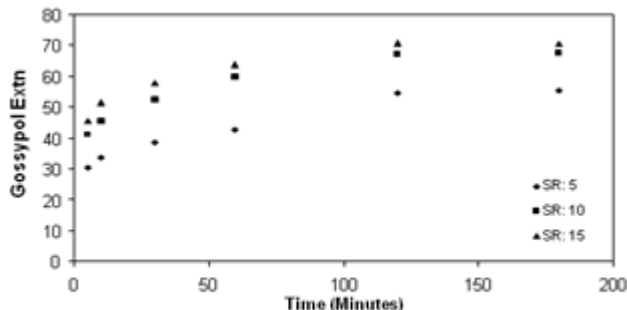


Figure 3: Effect of Time on Gossypol extraction by Acidic Ethanol at 308K

### 3.4 Effect of Temperature

The effect of temperature on gossypol extraction by ethanol acidified by 0.5M oxalic acid from DFS meal was studied. The results of percentage extraction at SR: 5 are shown in Figure 4. It indicates percentage extraction of gossypol increased from 55.37% to 78.67% with increase in temperature from 308 K to 343 K respectively. Earlier report shows that at SR:10 gossypol extraction increased from 67.5% to 86.22% with temperature increasing from 308 K to 343 K. The results obtained for SR: 15 indicate that gossypol extraction increased from 70.5% to 92.4 % under similar conditions, as shown in Figure 4.

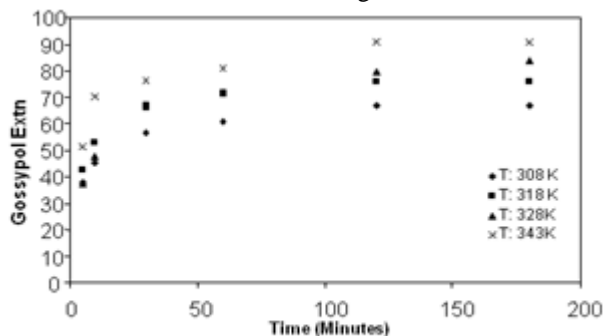


Figure 4: Effect of temperature on gossypol extraction with time by ethanol acidified by oxalic acid at SR:15

### 3.5 Kinetics of gossypol extraction:

Solid liquid extraction is a typical process consisting of two stages. In first stage the major part of the solute gets extracted quickly because of the dissolution and scrubbing of the surface solute caused by higher driving force of the fresh solvent and in the second stage extraction rate is slowed down due to slow diffusion of the remainder solute. To describe the process a typical second order reaction kinetics for the solid-liquid extraction process has been proposed by most of the researchers [18], [19], [20], [21], [22], [23], [24]. The rate of solute dissolution in solvent can be described by the following second order kinetics equation:

$$\frac{dC}{dt} = k(C_e - C)^2 \quad \text{----- (2)}$$

Where:

$k$  = The second-order extraction rate constant (mL/mg- $\square$  min)

$C_e$  = The concentration of gossypol in solvent at equilibrium (mg/mL)

The integrated form of Equation (2) obtained with initial condition at  $t = 0$  ,  $C_i = 0$ , could be written as

$$\frac{C}{t} = \frac{kC_e^2}{1 + C_e kt} \quad \text{----- (3)}$$

Now, when  $t \rightarrow 0$ , the LHS of Equation(3) will be initial extraction rate,  $E_o$ , and denominator of RHS will approach to 1.0 thus Equation (3) reduces to Equation (4)

$$E_o = kC_e^2 \quad \text{--- (4)}$$

Equation(3) and Equation(4) could be combined and rearranged to yield the following linear form:

$$\frac{1}{C} = \left( \frac{1}{E_o} \right) \frac{1}{t} + \frac{1}{C_e} \quad \text{-- (5)}$$

Thus initial extraction rate,  $E_o$ , the concentration of solute in the solution at equilibrium,  $C_e$  and the second order rate extraction constant,  $k$ , can be calculated from experimental data by plotting  $1/C$  vs.  $1/t$ .

Regression analysis of the experimental data yielded estimated results of gossypol extraction by ethanol acidified by tartaric acid from DF CSM at SR 5, 10 and 15 and temperature from 308 to 343K as shown in Table 1. The results show that for all the cases value of regression coefficient ( $R^2$ ) lies between 0.9259-0.9998 which indicates that the extraction process is well described by second order kinetics. The agreement of second order extraction model with experimental results supports the assumption that initially there is intense dissolution and scrubbing of gossypol from freely available glands in which maximum leaching takes place. The later slower rate occurs due to high diffusion resistance offered by solid surrounding the glands and the external diffusion, which is related to the soluble remainder gossypol. The variation of initial extraction rate,  $E_o$ , equilibrium concentration  $C_e$  and second order rate extraction constant,  $k$ , are given in Table 1.

**Table 1:** Kinetics parameters for gossypol extraction from DF CSM by ethanol acidified by oxalic acid

SR, mg/g	Temp, K	$C_e$	$E_i$	$k$	$R^2$
5	308	2.112	0.2196	0.0492	0.9992
5	318	2.687	0.3356	0.0464	0.9992
5	328	2.850	0.3788	0.0466	0.9994
5	343	2.912	0.6706	0.0791	0.9998
10	308	0.045	0.6762	324.91	0.9259
10	318	1.467	0.2766	0.1284	0.9998
10	328	1.664	0.1835	0.0662	0.999
10	343	1.757	0.3254	0.1054	0.9991
15	308	0.896	0.1584	0.1973	0.9995
15	318	1.012	0.2152	0.2101	0.9999
15	328	1.125	0.1256	0.0992	0.999
15	343	1.212	0.2308	0.1571	0.9988

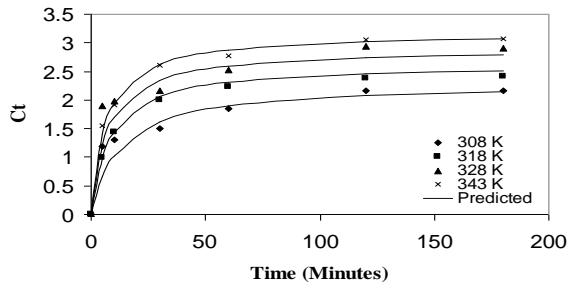
The following equation ( $R^2$  greater than 0.98) was found to give the estimated values of  $E_o$  and  $C_e$  using Table curve 3-D.

$$E_o = a + bT + c T^2 \text{----- (6)}$$

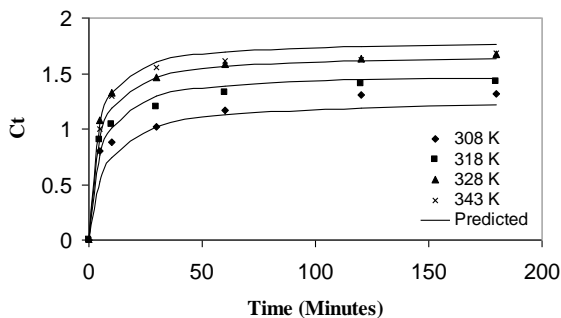
$$C_e = e + f S + g T + h S^2 + i T^2 + j S T \text{----- (7)}$$

Where:  $a = -19.25919$ ,  $b = 0.11157$ ,  $c = -0.0001573$ ,  $e = -42.37019$ ,  $f = 0.175583$ ,  $g = 0.254824$ ,  $h = 0.014916$ ,  $i = -0.00033768$ ,  $j = -0.001969$

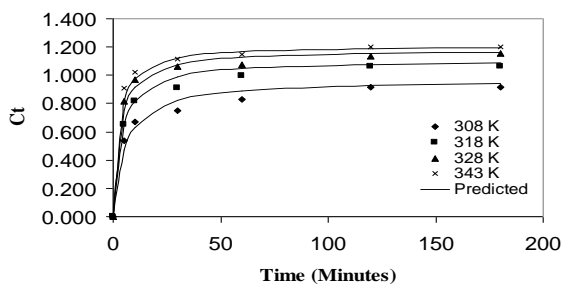
The gossypol concentration in ethanol was predicted using Equations (5) to (7) and results are shown in Figure 7 to Figure 9.



**Figure 7: Predicted kinetics parameters of gossypol extraction by Acidic Ethanol at SR:5**



**Figure 8: Predicted kinetics of gossypol extraction from by Acidic Ethanol at SR:10**



**Figure 9: Predicted kinetics of gossypol extraction by Acidic Ethanol at SR:15**

### 3.6 Thermodynamics of gossypol extraction from DFS by ethanol acidified by oxalic acid

Thermodynamics help in understanding the nature of extraction process. The Gibbs free energy change ( $\Delta G^\circ$ ) is an important criterion for spontaneity. If  $\Delta G^\circ$  value is negative reactions occur spontaneously at a given temperature. Gibbs free energy ( $\Delta G^\circ$ ), enthalpy change

( $\Delta H^\circ$ ) and entropy change ( $\Delta S^\circ$ ) are calculated using the following equations

$$K_e = \frac{C_e}{C_{se}} \text{---(8)}$$

$$\Delta G^\circ = -RT \ln K_e \text{---(9)}$$

$$\ln K_e = \left( -\frac{\Delta H^\circ}{R} \right) \frac{1}{T} + \frac{\Delta S^\circ}{R} \text{---(10)}$$

Where  $C_{se}$  is a concentration of gossypol in solid phase at equilibrium and could be obtained by material balance using Equation(1).  $R$  is the ideal gas constant ( $8.314 \text{ J mol}^{-1} \text{ K}^{-1}$ ), and  $T$  is the temperature (K). Eq. 10 is a Van't Hoff relation and Plot of  $\ln K_e$  vs  $1/T$  is used to obtain values of  $\Delta H^\circ$  and  $\Delta S^\circ$  from the slope and the intercept.  $\Delta G^\circ$  and  $\Delta H^\circ$  are in J/mol,  $\Delta S^\circ$  is in J/mol K

The values of  $K_e$ ,  $\Delta G^\circ$ ,  $\Delta H^\circ$  and  $\Delta S^\circ$  for extraction of gossypol using ethanol acidified with oxalic acid were calculated using Equations 8 to 10 and are given in Table 2. The values of  $\Delta G^\circ$  are positive for the gossypol extraction from DFS at all SR's and decrease from 4931.563 to 2884.028 J/mol at SR:5 ; from 5387.322 to 1642.225 J/mol at SR: 10; and from 6132.346 to 1611.135 J/mol at SR:15, with increase in temperature from 308 to 343K. The positive value for the Gibbs free energy for gossypol extraction from DFS confirms that the extraction process is feasible and non-spontaneous. The values of  $\Delta H^\circ$  calculated from the plot of  $\ln K_e$  versus  $1/T$  as shown in Table 2 are in the range 5325.45 to 14097.21 J/mol. The positive value of  $\Delta H^\circ$  indicate that the extraction process is endothermic. Positive value of entropy indicates that the process is irreversible.

**Table 2: Thermodynamics parameters for gossypol extraction from DF CSM by ethanol acidified by oxalic acid**

SR (ml/g)	T (K)	$K_e$	$\Delta G^\circ$ (J/mol)	$\Delta H^\circ$ (J/mol)	$\Delta S^\circ$ (J/mol K)
5	308	0.1457	4931.563	5325.45	18.775
5	318	0.2742	3420.776		
5	328	0.3352	2980.542		
5	343	0.3637	2884.028		
10	308	0.1219	5387.322	14097.2	45.855
10	318	0.1879	4419.901		
10	328	0.3601	2785.654		
10	343	0.5622	1642.225		
15	308	0.0912	6132.346	11348.6	37.484
15	318	0.2387	3786.923		
15	328	0.2655	3616.237		
15	343	0.5684	1611.135		

### 4. Conclusion

The results indicate that about 92.4 % gossypol can be removed by ethanol acidified with 0.5M oxalic acid from DFS meal at 318K, SR:15 compared to about 28 % with hexane (Saxena et. al.(2011) thereby making the meal suitable for food uses by blending with low protein cereals in such proportions that the free gossypol content is restricted to the safe limit of 450 ppm. At the same time, the separated gossypol can be further concentrated and purified

to fetch higher price due to its diversified use in medicinal, insecticide, pharmaceutical and plastic industry. A higher dose of DFS meal can be mixed in the cattle feed as a protein supplement after significant removal of gossypol.

The experimental data were analyzed and the kinetics and thermodynamics parameters of gossypol extraction process were obtained. This analysis shall be helpful in design of pilot scale study.

## References

- [1] Rathore K.S. "Reducing Gossypol in Cottonseed may Improve Human Nutrition", Texas Agricultural Experiment Station Texas A&M University 2007 [www.isb.vt.edu/articles/july0702.htm](http://www.isb.vt.edu/articles/july0702.htm)
- [2] Boatner C.H., and CM Hall, Oil & Soap **23**:123, 1946
- [3] Adams, R., T.A. Geissman and J.D. Edwards "Gossypol, a Pigment of Cottonseed". Chem. Review., 60: 555-574, 1960
- [4] Royce, H.D "Stability tests on fats containing oxidation accelerators and Inhibitors, Oil & Soap, **10**:123-125, 193,
- [5] Hron R.J. et. al. "The potential commercial aspects of gossypol", J.A.O. C.S. Vol. **64 (9)**: 1315-1319, 1987
- [6] Vadehra, D. V. et. al. "Antimicrobial activity of gossypol acetic acid", IRCS Medical Science **113(1)**, 10-11, 1985
- [7] Strax J.. "Cottonseed drug boosts cancer treatment in mice. [www.pсарisin.com/med/chemo/gossypol1004.htm](http://www.pсарisin.com/med/chemo/gossypol1004.htm), 2004
- [8] Smith F.H, Halverson J.O., "Extraction & Purification of gossypol From cottonseed meats", Oil & Soap, **Nov.1946**: 361-363, 1946
- [9] Chernenko, T.V. et. al. "Extraction of Gossypol" UDC 665.3/ 35 :665.1.03 Translated from Khimiya Prirodnykh Soedinenii, No.2: 157-160, 1980
- [10] Rama E.H., M.S. Narasinga Rao, "Gossypol removal and functional properties of Protein produced by extraction of glanded cottonseed with different solvents" J. Food Science, **49(4)**:1057-1060, 1984
- [11] Hron R J Sr and M S Kuk, "Acetone Extracted Cottonseed Meals without catty odor", J. Food Science **.54, (4)**, 1088-89 , 1989.
- [12] Bhowmick D.N. & Rebello D, Sponsored project: "Improved utilization of cottonseed meal by isopropanol extraction", Deptt. of Oils, U.I.C.T. Matunga, Mumbai, 2003
- [13] Kuk M, Tetlow R, Dowd M, "Cottonseed extraction with mixtures of Acetone & Hexane, Agricultural Research Service, USDA Research Project: New and Expanded Uses of Oilseed Products & By products., 2005
- [14] Dachery J.M. et. al. "Removal of gossypol from cottonseed by solvent extraction procedures" J. Am Oil Chem Society, 339-341, **Aug, 1952**
- [15] Junfeng Q., Zhi Y. , Haixian S. "Cogeneration bio diesel and non toxic cottonseed meal from cottonseed processed by two phase solvent extraction", Energy Conversion and Management, **51(12)**: 2750- 2756, 2010
- [16] Saxena D. K., Sharma S.K. and Sambhi S.S, Kinetics and Thermodynamics of cottonseed oil extraction., Grasas y aceites, **62 (2)**: 198-205, 2011
- [17] Hron R.J. et. al. , "Ethanol extraction of Oil, Gossypol and Aflatoxin from Cottonseed", J. Am Oil Chemist's Soc, **71(4)**: 417-421, 1994
- [18] Ho Y.-S. et al, "Kinetics and model building of leaching of water soluble compound of Tilia sapwood Separation and Purification Technology (**45**): 169-173, 2005

- [19] Meziane S, Kadi H and Lamrous O. "Kinetic study of oil extraction from olive foot cake". Grasas y Aceites, **57**: 175-179, 2006
- [20] Meziane S. and Kadi H, "Kinetics and thermodynamics of oil extraction from olive cake". J. Am Oil Chemist's Soc. **85(4)**: 391-396., 2008
- [21] Maria Y.L. et.al. "Extraction of Neem oil using n-hexane and ethanol: Studies on oil Quality, Kinetic & Thermodynamics", ARPN J. Engg. and Appl. Sc. **3 (3)**: 49-54, 2008
- [22] Rakotondramasy RL, Havet JL, Porte C and Fauduet H . "Solid-liquid extraction of protopine from fumaria officinalis L-
- [23] Analysis determination, kinetic reaction and model building", Sep.Purification Technol., **54(2)**, 253-261, 2007.
- [24] Sepidar S., Zurina Z. A., Yunus R. and Muhammad A." Extraction of Oil from Jatropha Seeds-Optimization and Kinetics, Am. J.Appl. Sci. **6(7)**, 1390-1395, 2009.
- [25] Wenjuan Q., Zhongli P, Haile M, "Extraction modeling and activities of antioxidants from pomegranate marc", J. Food Eng. **99**, 16-23, 2009.

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