Enzymatic Hydrolysis in Conjunction with Conventional Pretreatments to Soybean for Enhanced Oil Availability and Recovery

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ABSTRACT: Enzymatic hydrolysis of oilseeds prior to extraction has recently been shown to enhance the extractable oil in oilseeds and its recovery. This paper presents the results of optimizing the combination of enzymatic hydrolysis with one or more conventional pretreatments to soybean and of optimizing the hydrolysis parameters as they determine the mechanical extractability as well as the solvent extractability. Enzymatic hydrolysis in conjunction with flaking (dehulling inherent) and steam conditioning offered statistically the best pretreament combination for soybean at a 5% level of significance, enhancing the extractable oil content by about 4.8% of moisture-free sample. The optimal hydrolysis parameter values based on response surface analysis were: hydrolysis moisture content 23.22% wet basis, enzyme concentration 11.99 vol/wt, and incubation period 13.79 h. Over 99% of the total extractable oil released after hydrolysis was extractable within 16 h on a Soxhlet extractor.

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KEY WORDS: Enzymatic hydrolysis, extraction, hexane extraction, oilseeds pretreatment, soybean, soybean pretreatment.

Soybeans are now well established as a major oilseed crop in India, more so because they are a valuable source of protein-rich food as well. Conventionally, soybeans are deoiled by solvent extraction. Mechanical deoiling of soybeans, though not practiced commercially, is possible and has been proposed particularly under Indian conditions (1). Deoiling requires certain pretreatments. These include unit operations like dehulling, splitting, cracking/breaking, grinding or flaking, and cooking or steam conditioning (2). The purpose of pretreatments is to break the seed walls and release the oil for extraction.

Enzymatic hydrolysis has recently been shown to be another option for pretreatment. It opens up the cell walls through biodegradation and releases oil, thus serving the same purpose as the conventional pretreatments. In addition, it breaks up the complex lipoprotein and lipopolysaccharide molecules into simpler molecules, releasing extra oil which was otherwise nonextractable. Fullbrook (3) first observed this phenomenon in crude protein isolates from melon seeds and demonstrated its usefulness in ground soybean and rapeseed extraction. Enzymatic hydrolysis was later shown to enhance oil availability and/or recovery in various oilseeds pretreated through different combinations of conventional pretreatments, *viz.*, crushed and autoclaved soybean, sunflower, castor and cottonseeds (4), autoclaved canola flakes and seeds (5,6), broken soybean seed (7,8), soyflakes (8), soybean grits (9), and sunflower kernel halves (9). Further, enzymatic hydrolysis was shown to reduce the extraction time by Sosulski *et al.* (5) in canola flakes and by Kashyap *et al.* (8) in soyflakes and broken soybean seeds. Better oil recovery from mechanical deoiling of broken soybean seeds has been demonstrated by Smith *et al.* (7) using enzymatic hydrolysis. However, use of one or more conventional pretreatments along with the enzymatic hydrolysis was suggested to improve the oil recovery from mechanical deoiling of soybeans. Quality aspects including free fatty acid content, refractive value, and peroxide value of the oils so obtained have been reviewed by Dominguez *et al.* (10). The quality of oils obtained after enzyme treatment is good and unaffected. The oils are stable to rancidity, and their composition and structure are similar to untreated ones. A need still existed to establish which conventional pretreatment operations were required in conjunction with enzyme treatment for best effect. This information would help adapt the conventional process lines to enzyme-aided extraction process.

This research was therefore undertaken with an overall objective of optimizing the combination of enzymatic hydrolysis with one or more conventional pretreatments, optimizing the hydrolysis parameters, and determining the mechanical and solvent extractability.

MATERIALS AND METHODS

The experiments were conducted in three phases. In the first phase, the combination of conventional pretreatment unit operations—dehulling, size reduction, and thermal treatment—in conjunction with enzymatic hydrolysis was optimized for maximum extractable oil released. The size reduction pretreatments were splitting, breaking, flaking, and grinding with and

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TABLE 1
Second-Order Central Composite Rotatable Design for Three Variables at Five Levels of Each Variable[a]

Coded variables			Number of combinations	Number of replications	Number of experiments
X_1	X_2	X_3			
0	0	0	1	6	6
±1	±1	±1	8	2	16
±1.682	0	0	2	2	4
0	±1.682	0	2	2	4
0	0	±1.682	2	2	4

[a]Total number of experiments = 34.

without hulls. The thermal pretreatments were oven cooking and steam conditioning. Steam conditioning of ground soybeans was not considered because it resulted in a high-moisture paste which, upon drying, became a hard cake. The enzymatic hydrolysis was performed at about the conditions reported optimum for broken soybean seeds and soyflakes by Kashyap *et al.* (8) and Smith *et al.* (7). Moisture content during hydrolysis was 24% w/w, enzyme concentration 12% vol/wt, incubation period 13.5 h, incubation temperature 45°C, and *Aspergillus fumigatus* was the source microorganism for enzyme (enzyme activity 0.5 to 1.4 IU/mL). Soybeans so treated were analyzed in triplicate for extractable oil released by Soxtec Extractor (Tecator-1043, Hoganas, Sweden) using petroleum ether as solvent and carrying out the extraction at 105°C. The boiling and rinsing times were kept at 30 and 120 min, respectively, as this step extracted almost all of the oil in comparison with manufacturer's specifications. Prior to extraction, moisture was adjusted to 10% w/w, and the samples were ground to manufacturer's specifications. The data were statistically analyzed to establish the best combination.

For the best combination of pretreatments, the enzymatic hydrolysis parameters (hydrolysis moisture content, enzymatic concentration, and incubation period) were optimized for maximum extractable oil in the second phase. The experimental design was second-order central composite rotatable orthogonal design with full replicates (11) in three variables at five levels. The ranges of parameter values investigated were 19.64 to 26.36% w/w hydrolysis moisture content, 4.27 to 17.73% vol/wt enzyme concentration, and 5.27 to 18.7 h incubation time. The data on increase in extractable oil released due to pretreatments, as determined on a Soxtec Extractor, were analyzed using the multiple regression technique, and the response surface models were developed. The optimal values of hydrolysis parameters were calculated by partially differentiating the response function. The maximized extractable oil released at optimal conditions as predicted by the models was verified experimentally. In the third phase, mechanical expellability and solvent extractability of soybeans processed through optimal pretreatments were investigated. Laboratory Carver Press (Freed S. Carver Inc., Menomonee Falls, WI) and Soxhlet apparatus were used, respectively. The extraction and pressing parameters were kept constant at the levels reported in literature (7,8).

RESULTS AND DISCUSSION

Optimization of the combination of conventional unit pretreatment in conjunction with enzymatic hydrolysis. The experimental design and variables used in these experiments are shown in Tables 1 and 2. Increase in the extractable oil varied, from about 0.5 to 4.2% of moisture-free sample (2.3 to 19.1% of total oil), with the pretreatments used (Table 3). The increase of about 1.7% due to enzymatic hydrolysis of uncooked broken soybean seeds was comparable to the 1.4% observed by Smith *et al*. (7) and 1.5% by Kashyap *et al.* (8) under somewhat different hydrolysis conditions. Similarly, the 1.9% increase due to enzymatic hydrolysis of uncooked soyflakes was in conformity with the 1.95% increase reported by Kashyap *et al.* (8). Dominguez *et al.* (9) also reported comparable increases, 0.7 to 2.5% dry basis (3.7 to 12.1% of total oil) in heat-treated soybean grits of 1.2 to 0.8 mm, depending upon the enzyme used. The combination of enzymatic hydrolysis with flaking and steam-conditioning pretreatments resulted in a maximal increase in the extractable oil over that in untreated soybeans (dehulled in this case). The increase was 4.23% of moisture-free sample, and was significantly higher than that in all other pretreatment combinations at a 5% level of significance (Table 4). The next best pretreatment combinations of enzymatic hydrolysis in conjunction with flaking and cooking (2.93% oil increase), often used commercially, and with breaking and steam conditioning (2.90% oil increase) were significantly better than the remaining combina-

TABLE 2
Coded and Uncoded Values of Variables

Code	Moisture content during hydrolysis (%, w/w), X_1	Enzyme concentration (%, vol/wt), X_2	Incubation period (h), X_3
+1.682 Star/axial point	26.364	17.728	18.728
+1 Corner point	25	15	16
0 Point	23	11	12
−1 Corner point	21	7	8
−1.682 Star/axial point	19.636	4.272	5.272

TABLE 3
Increase in Extractable Oil Attributed to Enzymatic Hydrolysis of Soybean Seeds

Combination of pretreatment unit operations		Extractable oil after pretreatment (%, moisture-free basis)	Increase[a] in extractable oil due to pretreatment (%, moisture-free basis)
Thermal treatment	Size reduction		
None	None	21.94 ± 0.05[b]	0.49 ± 0.05
	Splits	22.92 ± 0.03	1.42 ± 0.03
	Broken	23.24 ± 0.09	1.73 ± 0.09
	Flakes	23.40 ± 0.11	1.90 ± 0.11
	Ground soybeans with hulls (passing through 0.4-mm sieve)	22.17 ± 0.15	0.71 ± 0.15
	Ground soybean with hulls	22.22 ± 0.12	0.72 ± 0.12
Oven cooking (90°C, 75 min, 10% w/w moisture content, closed container)	None	22.32 ± 0.04	0.82 ± 0.04
	Splits	22.79 ± 0.10	1.29 ± 0.10
	Broken	23.51 ± 0.08	2.01 ± 0.08
	Flakes	24.44 ± 0.13	2.93 ± 0.13
	Ground soybean with hulls	22.16 ± 0.13	0.70 ± 0.13
	Ground soybean	22.29 ± 0.02	0.79 ± 0.02
Steam conditioning (2-min exposure at atmospheric pressure)	None	22.42 ± 0.09	0.97 ± 0.09
	Splits	23.41 ± 0.05	1.91 ± 0.05
	Broken	24.41 ± 0.10	2.90 ± 0.10
	Flakes	25.73 ± 0.26	4.23 ± 0.25

[a]Calculated by subtracting the oil content prior to treatment from extractable oil content after the treatment. Oil content of untreated soybeans with hulls (average of three replications) was 21.46% of moisture-free sample. Oil content of untreated soybean without hulls was 21.51%.
[b]Average of three replications ± standard deviation.

tions although inferior to the best combination. These offered the advantage of eliminating the pretreatment operations of steaming and flaking, respectively, at a sacrifice of about 1.3% oil.

Optimization of enzymatic hydrolysis parameters in the optimal combination of pretreatment unit operation. Extractable oil released in steam-conditioned soyflakes was affected by enzyme concentration, moisture content during hydrolysis, and time of incubation (Table 5). The average increase in extractable oil due to pretreatments was analyzed by response surface methodology (12) employing multiple regression technique. The following equations for response surface were fitted, and the adequacy was tested employing the F test. If the response function was found adequate, the coefficients of different terms were examined for their significance employing Student's *t*-test.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 \quad [1]$$

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1^2 + b_5X_2^2 + b_6X_3^2 \quad [2]$$

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_7X_1X_2 + b_8X_1X_3 + b_9X_2X_3 \quad [3]$$

TABLE 4
Analysis of Variance (ANOVA) for Increase in the Release of Extractable Oil Due to Conventional Unit Pretreatments in Conjunction with Enzymatic Hydrolysis of Soybean Seeds

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value (calculated)[a]
Between sample	48.51	15	3.23	300.93
Error	0.34	32	0.01	
Total	48.85	47		

[a]Table F-value with degrees of freedom (Df) (15, 32, 0.05%) = 2.01. Critical difference = 0.173. Note: Effect of pretreatment combinations is significant because $F_{calc} > F_{table}$.

TABLE 5
Oil Availability in Steam-Conditioned and Enzymatically Hydrolyzed Soyflakes as Affected by Enzymatic Hydrolysis Parameters

Enzymatic hydrolysis parameters			Extractable oil[a] after pretreatment (%, moisture-free sample)	Increase in extractable oil[b] (%, moisture-free sample)
X_1	X_2	X_3		
23	11	12	26.34	4.83
25	15	16	26.04	4.54
25	15	8	25.62	4.11
25	7	16	25.57	4.07
25	7	8	25.32	3.81
21	15	16	25.81	4.30
21	15	8	25.60	4.09
21	7	16	25.56	4.05
21	7	8	25.62	4.12
26.36	11	12	25.75	4.25
19.63	11	12	25.70	4.20
23	17.72	12	25.63	4.12
23	4.27	12	25.11	3.60
23	11	18.72	26.13	4.62
23	11	5.27	25.81	4.30

[a]Each entry represents the average of two replications of treatment and in each three replications of oil determination except the first which is an average of six replications of treatment.
[b]Calculated by subtracting the extractable oil before the treatment from extractable oil after the treatment. Extractable oil before the treatment (i.e., in unhydrolyzed uncooked soyflakes) was 21.5% moisture-free sample. X_1 = % hydrolysis moisture; X_2 = % enzyme concentration; X_3= incubation time, h.

TABLE 6
ANOVA of Equation 5 for Increase in Extractable Oil

Sources of variation	Sum of squares	Degrees of freedom	Mean sum of squares	F-value[a] (calculated)
Regression	1.28	8	0.16	160.94
Residual	0.06	6	0.001	
Total	1.29	14		

[a]Table F-value with Df (8, 6, 0.95) = 4.15. See Table 4 for abbreviation.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_1^2 + b_5X_2^2 + b_6X_3^2 + b_7X_1X_2 + b_8X_1X_3 + b_9X_2X_3 \quad [4]$$

where Y = increase in extractable oil released, % of moisture-free sample; X_1 = moisture content during hydrolysis, % w/w; X_2 = enzyme concentration, % enzyme volume/sample weight; and X_3 = incubation period, h.

The adequate models were further compared based on their correlation coefficient, standard error, and residual analysis (13). The model describing the response surface of increase in extractable oil released as affected by enzymatic hydrolysis parameters in steam-conditioned soyflakes was thus obtained (Tables 6 and 7):

$$Y = -23.6631 + 2.317\,X_1 + 0.2726\,X_2 - 0.05449\,X_1^2 - 0.02157\,X_2^2 - 8.403 \times 10^{-3}\,X_3^2 + 8.4437 \times 10^{-3}\,X_1X_2 - 8.1707 \times 10^{-3}\,X_1X_3 + 3.5044 \times 10^{-3}\,X_2X_3 \quad [5]$$

The model showed that moisture content during hydrolysis (X_1), enzyme concentration (X_2), and incubation period (X_3) interacted with each other during the hydrolysis process and were not independent of each other. Sosulski *et al.* (5) had also reported interaction between moisture content during hydrolysis and enzyme concentration. The standardized partial regression coefficients (β) obtained in the multiple regression analysis indicated that hydrolysis moisture content had a greater interaction with enzyme concentration and incubation period as compared to the interaction between enzyme concentration and incubation period.

The predicted three-dimensional response surfaces for the increase in extractable oil in steam-conditioned soyflakes were generated by computer as a function of two parameters at a time while maintaining the third parameter at its center point value (Fig. 1). The increase in extractable oil first increased with increase in either parameter value and then decreased, showing the existence of an optimum for maximum increase in oil within the parameter ranges investigated. Optimal enzymatic hydrolysis conditions for maximum increase in extractable oil as calculated by partially differentiating Equation 5 with respect to each parameter and setting the result equal to zero were: moisture content during hydrolysis, X_1 = 23.22% w/w; enzyme concentration, X_2 = 11.99% enzyme vol/wt; incubation period, X_3 = 13.79 h. At the optimum hydrolysis parameter values, the maximum increase in extractable oil predicted by response function was 4.88% moisture-free basis. The predictions from the foregoing statistical analysis were physically validated by enzymatic treatment of steam-conditioned soyflakes at the predicted optimal hydrol-

TABLE 7
Regression Estimate and Constants of Equation 5 for Increase in Extractable Oil

Estimator	Estimate	Standard error	Beta	*t* Values[a] (calculated)
b_0	−23.66	1.63		−14.51
b_1	2.32	0.14	15.07	16.94
b_2	0.27	0.04	3.55	7.38
b_4	−0.05	3.05E − 03	−16.32	−17.87
b_5	−0.02	7.62E − 04	−6.26	−28.30
b_6	−8.40E − 03	6.93E − 03	2.60	6.05
b_7	8.44E − 03	1.39E − 03	2.60	6.05
b_8	8.17E − 03	7.60E − 04	2.53	10.75
b_9	3.05E − 03	6.84E − 04	0.75	5.12

[a]*t*-Table (6, 0.95) = 1.943.

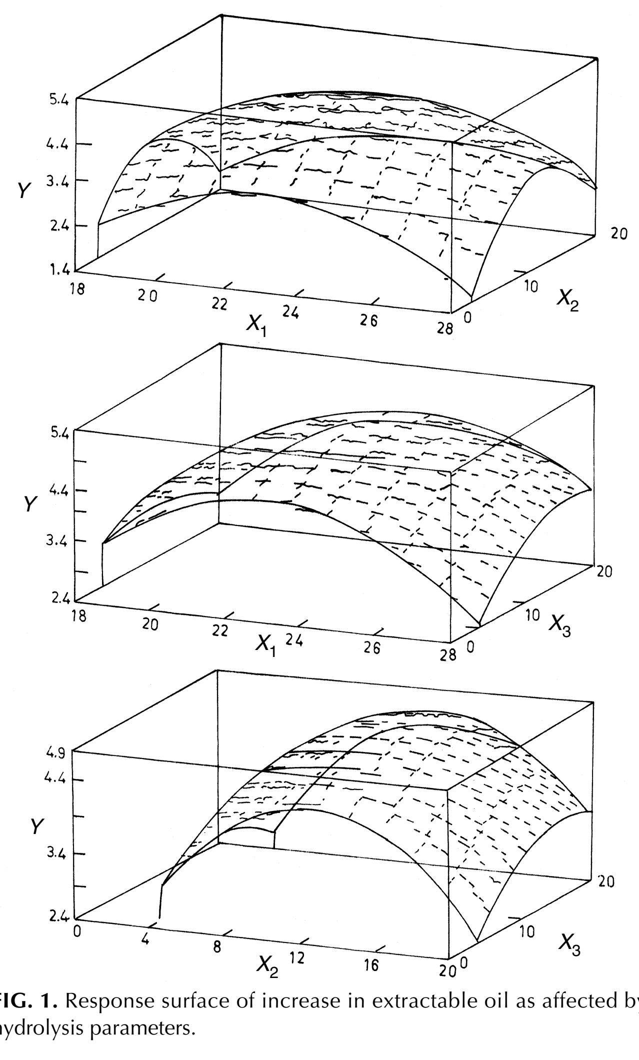

FIG. 1. Response surface of increase in extractable oil as affected by hydrolysis parameters.

ysis conditions. The increase in extractable oil was observed to be 4.85% on moisture-free basis as against the predicted value of 4.88%, thereby confirming the predicted optima for enzymatic hydrolysis.

Further, solvent extractability and expellability of optimally treated soyflakes were determined. The Soxhlet extraction for 16-, 20-, and 24-h extraction periods showed that over 99% of total extractable oil after hydrolysis was extracted within 16 h. Similar extraction time was reported by Kashyap *et al*. (8) for extraction of unsteamed soyflakes after hydrolysis. The oil recovery was 26.2% of moisture-free sample weight.

Mechanical pressing with a Carver Press resulted in an oil recovery of 16.7% of moisture-free sample weight (63.4% of the total extractable oil after hydrolysis) from steam-conditioned hydrolyzed soyflakes. Smith *et al*. (7) reported 15.8% oil recovery from enzymatically hydrolyzed broken soybean seeds without steaming. This showed that the enhanced oil recovery resulted primarily from extra release of oil rather than enhanced expellability. Note that the 63.4% oil recovery in the Carver Press was under static press conditions at room temperature. Much higher oil recovery would be expected in an actual screw expeller owing to dynamic pressing and higher operating temperature. Sosulski and Sosulski (6) have reported an oil recovery of 90 to 93% for enzyme-treated canola seeds in a laboratory expeller as against 72% in an untreated sample.

In conclusion, enzyme treatment is most effective when applied in conjunction with flaking and steaming operations at the optimal conditions determined in this study. Commercially, the pretreatments generally used in soybean ex–traction are flaking and cooking (14) and sometimes flaking and steam conditioning (15). The process lines having flaking and steaming operations can therefore be directly adapted to the enzyme treatment. The process lines employing flaking and cooking can either be adapted directly to enzyme processing by marginal sacrifice in the enhancement of oil recovery, or the cooking operation can be modified to steam conditioning for the best effect of enzyme treatment. Mechanical expelling of soybean oil, which is not used commercially due to low oil recovery, also may be improved with enzyme treatment. However, actual expeller runs would need to be conducted and expeller operation optimized before commercial application.

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REFERENCES

1. Singh, B.P.N., and Y.C. Agrawal, *Soybean Processing and Utilization in India,* edited by N. Ali, A.P. Gandhi, and T.P.Ojha, Central Institute of Agricultural Engineering, Bhopal, India, 1988, pp. 414–423.
2. Galloway, J.P., Cleaning, Cracking, Dehulling, Decorticating and Flaking of Oil-bearing Materials, *J. Am. Oil Chem. Soc. 53*:271–274 (1976).
3. Fullbrook, P.D., The Use of Enzymes in the Processing of Oilseeds, *Ibid. 60*:476–478 (1983).
4. Bhatnagar, S., and B.N. Johri, Microbial Enzymes in the Processing of Oilseeds, *Curr. Sci. 56*:775–776 (1987).
5. Sosulski, K., F.W. Sosulski, and E. Coxworth, Carbohydrase Hydrolysis of Canola to Enhance Oil Extraction with Hexane, *J. Am. Oil Chem. Soc. 65*:357–361 (1988).
6. Sosulski, K., and F.W. Sosulski, Enzyme-Aided vs. Two-Stage Pressing of Canola: Technology, Product Quality and Cost Analysis, *Ibid. 70*:825–829 (1993).
7. Smith, D.D., Y.C. Agrawal, B.C. Sarker, and B.P.N. Singh, Enzymatic Hydrolysis Pretreatment for Mechanical Expelling of Soybeans, *Ibid. 70*:885–890 (1993).
8. Kashyap, M.C., Y.C. Agrawal, B.C. Sarker, and B.P.N. Singh, Response Surface Analysis of Enzyme-Aided Extraction of Soybean for Enhanced Oil Recovery, *J. Food Sci. Technol. 34*:386–390 (1997).
9. Dominguez, H., M.J. Nunez, and J.M. Lema, Oil Extractability from Enzymatically Treated Soybean and Sunflower: Range of Operational Variables, *Food Chem. 46*:277–286 (1993).
10. Dominguez, H., M.J. Nunez, and J.M. Lema, Enzymatic Pretreatment to Enhance Oil Extraction from Fruits and Oil Seeds: A Review, *Ibid. 49*:271–286 (1994).
11. Myers, R.H., *Response Surface Methodology,* Allyn and Bacon, Boston, 1976, pp. 126–166.
12. Johnson, N.L., and F.C. Leone, *Statistics and Experimental Design in Engineering and the Physical Sciences,* 2nd edn., John Wiley & Sons, New York, 1977, Vol. II, pp. 909–975.
13. Shaw, G., and D. Wheeler, *Statistical Techniques in Geographical Analysis,* John Wiley & Sons, New York, 1985, pp. 183–187.
14. Becker, K.W., Processing of Oilseeds to Meal and Protein Flakes, *J. Am. Oil Chem. Soc. 48*:299–304 (1971).
15. Norris, F.A., Extraction of Fats and Oils, in *Bailey's Industrial Oil and Fat Products,* 3rd edn., edited by D. Swern, Interscience Publishers, New York, 1964, pp. 637–692.

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