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ARTICLE

Corn Steep Liquor as a Nutrition Adjunct for the Production of *Aspergillus niger* Lipase and Hydrolysis of Oils Thereof

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Corn steep liquor (CSL) has been used as a nutrition adjunct for the production of an extracellular lipase from *Aspergillus niger*, which has immense importance as an additive in laundry detergent formulations. A five-level four-factorial central composite design was chosen to determine the optimal medium components with four critical variables, namely, CSL, $NH_4H_2PO_4$, Na_2HPO_4, and sesame oil, that were found to be influential for lipase production by the classical one-factor-at-a-time method. The model suggested that all of the factors chosen had a significant impact on lipase production, and the optimum values of the influential parameters were CSL, 2.0%, w/v; $NH_4H_2PO_4$, 0.05%, w/v; Na_2HPO_4, 0.75%, w/v; and sesame oil, 2.0%, w/v, with an activity of 26.7 U/mL at 48 h and 30 °C, which was 2.16-fold higher than the initial activity (12 U/mL) obtained by the conventional one-factor-at-a-time method. Furthermore, the enzyme has good potential for the hydrolysis of vegetable oils and fish oils, and a hydrolytic ratio of 88.73% was obtained with palm oil at 48 h. The utilization of CSL and sesame oil for lipase production from *A. niger* makes the process green, because both are renewable substrates and economically viable at an industrial scale.

KEYWORDS: Lipase; *Aspergillus niger*; corn steep liquor; sesame oil; hydrolysis

INTRODUCTION

Corn steep liquor (CSL), a byproduct of the corn wet-milling industry, is used as an ingredient in animal feed and as a nutrient supplement for microorganisms in diverse industrial fermentation processes. It is a mixture consisting of water-soluble extracts of corn soaked (steeped) in water, composed entirely of natural amino acids, minerals, vitamins, reducing sugars, organic acids, enzymes, and other elemental nutrients, which are excellent source of nutrients for microorganisms (*1*, *2*). The most important application of CSL in microbiology was initially discovered by Moyer and Coghill (*3*), who noticed that the addition of CSL to the liquid medium greatly increased the yields of penicillin from *Penicillium notatum*. Later, Gern et al. (*4*), Lotfy et al. (*5*), Essamri et al. (*6*), and Schroeder (*7*) reported that supplementation of CSL to the growth medium increased the mycelial growth of *Pleurotus ostreatus*, *Aspergillus niger*, *Rhizopus oryzae*, and *Aspergillus parasiticus*, respectively, because CSL also adjusts the trace metal balance in the fermentation medium.

CSL is an inexpensive, readily available material (*8*) and is used as a complex nitrogen source for the production of various metabolites including lipase (*6*, *9*−*11*), xylanase (*12*), uricase (*13*), cephalosporin C (*14*), citric acid (*5*), succinic acid (*15*), β-glucans (*4*), and bioethanol (*16*). In the industrialization of fermentation processes, the nutrient cost of the medium is one of the most important factors (*16*), and supplementation of organic nitrogen source such as yeast extract or meat extract is too costly for commercial production of enzymes and biofuels, because it could govern 50% of the overall medium cost (*17*). Therefore, exploring locally available sources of organic nitrogen from renewable agro-industrial residues such as CSL is essential for the production of lipases from microorganisms, in order to make the process viable and green.

Lipases are ubiquitous enzymes of considerable physiological significance and industrial potential. Interest in the production of microbial lipases has increased in recent decades, because they find promising applications in the production of pharmaceuticals, detergents, cosmetics, leather, and foods and in other organic syntheses (*9*, *18*, *19*). Among microorganisms, molds are known to be more potent lipase producers, because they produce lipase both by solid substrate and by submerged fermentation (*20*, *21*). Hence, it is worthwhile to optimize the fermentation medium, which affects the product yield and volumetric productivity of these enzymes. Medium optimization by the conventional one-factor-at-a-time method is time-consuming and inept at reaching the true optimum, because it does not include interaction effects among variables. The statistical design of experiments is an organized approach that yields more reliable information per experiment than conventional approaches. Response surface methodology (RSM) has also been used by several researchers to investigate the optimal production of lipase for various applications (*22*−*24*); recently, we have shown the application of *A. niger* lipase as an additive in detergent formulation by RSM (*25*), wherein the lipase was produced from a liquid medium containing meat extract as the sole nitrogen source and olive oil as

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an inducer. In this study, we have used central composite rotatable design (CCRD), a tool of RSM, to determine the optimal conditions for production of an extracellular lipase from *A. niger* MTCC 2594 with an economically feasible medium using CSL, an important nutrition adjunct as well as an agro-industrial residue, phosphates, and sesame oil, an inducer, which were found to be optimum by the classical one-factor-at-a-time method. Furthermore, the lipase was partially purified and evaluated for the hydrolysis of vegetable oils and fish oils, which are industrially and economically important for the production of fatty acid esters and n-3 series of polyunsaturated fatty acids, respectively.

MATERIALS AND METHODS

Materials. All of the chemicals used in the present study were of AR grade and purchased from Hi-Media Limited and SD Fine Chemicals Limited, Mumbai, India. Corn steep liquor and fish oils were obtained as gifts from Anil Products Limited, Mumbai, India, and Raj Fisheries Limited, Mangalore, India, respectively. The other ingredients of the media, namely, sucrose, sodium chloride, sesame oil, palm oil, sunflower oil, soy oil, and olive oil, were purchased from local markets in Chennai, India.

Inoculum and Culture Conditions. A fungal strain of *A. niger* MTCC 2594 isolated in our laboratory was used in the present study, and it was maintained on Czapek Dox agar slants at 4 °C. The spore suspension for inoculation was prepared by adding 2 mL of sterile distilled water to the culture slant, and the spores were dislodged using an inoculation needle under aseptic conditions. A spore suspension containing 4.3×10^8 spores/mL was used as an inoculum. Lipase production was carried out in 250 mL Erlenmeyer flasks, each containing 40 mL of the sterile production medium of pH 7.0. The medium was inoculated with 240 μL of spore suspension and grown for 48 h on a rotary shaker at 30 °C and 120 rpm. After growth, the biomass was removed by filtration, and the cell-free supernatant was used as crude enzyme preparation. All of the experiments were carried out in batches, and the results are expressed as the mean of triplicates.

Medium Optimization with CSL as Nutrition Adjunct. Lipase production was initially carried out in the production medium of Kamini et al. (*26*) with the following composition (%, w/v): meat extract, 1; urea, 0.5; sucrose, 0.5; $MgSO_4 \cdot 7H_2O$, 0.05; KH_2PO_4, 0.1; Na_2HPO_4, 0.3; and olive oil, 1. To develop a low-cost medium, optimization studies were investigated by the classical one-factor-at-a-time method using CSL (0.5−5.0%, w/v), urea, sucrose, sodium chloride, various inorganic nitrogen sources [NH_4NO_3, $(NH_4)_2SO_4$, $NH_4H_2PO_4$, and $NaNO_3$], phosphates (KH_2PO_4 and Na_2HPO_4), and inducers (olive oil, palm oil, coconut oil, sesame oil, and sunflower oil) for the production of lipase.

Enzyme Assay. Lipase activity was determined according to the method of Yamada et al. (*27*) using olive oil as substrate. One unit of activity was defined as the amount of enzyme releasing 1 μmol of free fatty acid in 1 min at pH 7.0 and 37 °C.

Factorial Design. On the basis of the results obtained by the classical method, four critical variables were selected for optimization by RSM. A five-level four-factorial CCRD was employed in this study for the factors *A* (CSL), *B* ($NH_4H_2PO_4$), *C* (sesame oil), and *D* (Na_2HPO_4) with an α value of ±1.414. The relationship between the variation of the response, Yc (lipase activity, U/mL) and the variation of factors *A*−*D* is represented by a second-order mathematical model using the equation

$$\begin{aligned} \mathrm{Yc} = {} & \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 \text{ (intercept and main effects)} \\ & + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 + \beta_{33} {X_3}^2 + \beta_{44} {X_4}^2 \text{ (interactions)} \\ & + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 \\ & + \beta_{34} X_3 X_4 \text{ (quadratic effects)} \end{aligned}$$

where Yc was the response calculated by the model and X_1, X_2, X_3, and X_4 were the coded variables corresponding to factors *A*, *B*, *C*, and *D*, respectively. Coding was required, because the factors were expressed in different units, and β_0 represented the regression coefficient at the center. β_1, β_{11}, and β_{12} were coefficients estimated by the model, which represented the linear quadratic and interactive effects of X_1, X_2, X_3, and X_4 factors on the response, respectively. The treatment combinations of CCRD were allocated in three blocks, and each block had 10 runs. The first two blocks each had eight factorial points and two center points. The last block had eight axial points and two center points. Thus, in total, the experimental setup consisted of 30 trials, and the value of the dependent response was the mean of triplicates.

Data Analysis. The data from the experiments performed were analyzed and interpreted using Design Expert software package (version 7.0.3, Stat-Ease Inc., Minneapolis, MN). Three main analytical steps: analysis of variance (ANOVA), regression analysis, and plotting of response surface, were performed to establish an optimum condition for lipase production.

Hydrolysis of Various Oils by *A. niger* Lipase. Crude culture filtrate containing lipase of *A. niger* produced by RSM was subjected to ammonium sulfate precipitation (80% saturation). The resulting precipitate was dissolved in 0.01 M phosphate buffer (pH 7.0) and dialyzed against the same buffer. The partially purified lipase was lyophilized and used for the hydrolysis of vegetable oils and fish oils.

Hydrolytic reactions were carried out in series of 100 mL Erlenmeyer flasks containing 1 g of oil, 15 mL of 0.1 M phosphate buffer (pH 7.0), and 50 U of lipase (81.74 U/mg of protein) and incubated at 30 °C for 120 h with shaking at 120 rpm. Samples were taken at 24 h intervals, and reaction was stopped by the addition of 20 mL of ethanol; the free fatty acids liberated were titrated with 0.1 N KOH. A control was carried out similarly, except the enzyme solution was added after the addition of ethanol. The control value was subtracted from the experimental value, and the acid value was calculated. The hydrolysis ratio was calculated by the following equation:

$$\text{hydrolysis ratio (\%)} = \text{acid value/saponification value} \times 100$$

The effects of lipase concentration, substrate/buffer ratio, and use of additives and solvents in the reaction mixture were also determined for palm oil to achieve maximum hydrolysis. The fatty acid composition of the hydrolyzed palm oil was estimated by esterification of the fatty acids to their respective methyl esters as described by Kanya et al. (*28*). The methylated esters were quantified by gas chromatography (Hewlett-Packard 6890) connected to a BPX-70 column (50 m × 0.22 mm × 0.25 μm; J&W Scientific, Folsom, CA). Helium was used as the carrier gas. The column temperature was set at 150 °C, raised to 240 °C at the rate of 7.5 °C/min, and held for 10 min. The temperatures of the injector and detector were set at 250 and 300 °C, respectively. Identifications of the methyl esters were made by comparison of retention time along with methyl heptadecanoate as an internal standard.

RESULTS AND DISCUSSION

The production of lipase from *A. niger* MTCC 2594 in submerged fermentation was reported earlier by Kamini et al. (*26*). Economic analysis of the above medium revealed that addition of meat extract alone to the medium contributes about 66.8% of medium cost. Therefore, attempts were made to make the process economically viable by using an inexpensive nutrition adjunct, CSL, because it is an excellent source of nitrogen and carbon for most of the microorganisms (*8*) and could replace the expensive yeast extract and polypeptone as reported by Gern et al. (*4*) and Lee et al. (*15*), respectively. By conventional one-factor-at-a-time approach, an optimal lipase activity of 12 U/mL was obtained at 48 h by replacing meat extract with 1.25% CSL, in a medium supplemented with sucrose, 0.5%; NaCl, 0.5%; urea, 0.25%; $NH_4H_2PO_4$, 0.125%; KH_2PO_4, 0.1%; Na_2HPO_4, 1.0%; and sesame oil, 1.5% (data not shown). This lipase activity was comparatively higher than the lipase activity of *Fusarium oxysporum*, which showed 5 U/mL of lipase activity after 72 h of fermentation in a medium containing 8% CSL (*29*), whereas a higher lipase activity (20 U/mL) was reported for *Geotrichum* sp. in a medium with 13% CSL (*30*). Such a higher concentration of CSL may affect the downstream processing of the enzyme, and in the case of *Rhizopus* sp., addition of 5% CSL to the medium

Table 1. Experimental Range of Variables for the Central Composite Design

variable	coded symbol	range of variables (%, w/v) low (−1)	mid (0)	high (+1)
CSL	A	0.50	1.25	2.00
$NH_4H_2PO_4$	B	0.05	0.125	0.20
sesame oil	C	1.00	1.50	2.00
Na_2HPO_4	D	0.75	1.00	1.25

Table 2. Central Composite Rotatable Design (CCRD) Matrix of Independent Variables and the Corresponding Experimental and Predicted Values of Lipase Production by *Aspergillus niger*

	medium components (%, w/v)				lipase activity (U/mL)	
run	CSL	$NH_4H_2PO_4$	sesame oil	Na_2HPO_4	exptl	predicted
1	0.50	0.05	1.00	0.75	6.40	6.09
2	2.00	0.05	1.00	0.75	22.30	22.55
3	0.50	0.20	1.00	0.75	8.70	8.10
4	2.00	0.20	1.00	0.75	23.50	22.92
5	0.50	0.05	2.00	0.75	12.40	12.5
6	2.00	0.05	2.00	0.75	26.78	25.42
7	0.50	0.20	2.00	0.75	14.40	14.72
8	2.00	0.20	2.00	0.75	17.70	18.50
9	0.50	0.05	1.00	1.25	3.75	2.93
10	2.00	0.05	1.00	1.25	20.10	19.83
11	0.50	0.20	1.00	1.25	11.31	12.72
12	2.00	0.20	1.00	1.25	20.60	20.48
13	0.50	0.05	2.00	1.25	13.40	14.03
14	2.00	0.05	2.00	1.25	19.30	19.89
15	0.50	0.20	2.00	1.25	16.80	16.54
16	2.00	0.20	2.00	1.25	20.40	20.76
17	1.25	0.13	1.50	1.00	12.59	12.03
18	1.25	0.13	1.50	1.00	11.41	12.03
19	1.25	0.13	1.50	1.00	14.30	13.90
20	1.25	0.13	1.50	1.00	13.71	13.90
21	0.19	0.13	1.50	1.00	6.45	6.13
22	2.31	0.13	1.50	1.00	20.50	20.75
23	1.25	0.02	1.50	1.00	5.69	6.55
24	1.25	0.23	1.50	1.00	9.51	8.58
25	1.25	0.13	0.79	1.00	15.91	16.65
26	1.25	0.13	2.21	1.00	22.20	21.38
27	1.25	0.13	1.50	0.65	11.79	12.77
28	1.25	0.13	1.50	1.35	13.19	12.13
29	1.25	0.13	1.50	1.00	11.14	11.54
30	1.25	0.13	1.50	1.00	11.63	11.54

completely inhibited the growth and synthesis of lipase (*21*). However, a maximum lipase activity of 12 U/mL was obtained with our strain using 1.25% CSL, and further statistical optimization of the medium components was carried out by CCRD.

CCRD Model Fitting and ANOVA. The experimental domain depicting the levels for each selected factor is given in **Table 1**. The levels of the factors were chosen on the basis of the results obtained from the conventional one-factor-at-a-time approach. The experiments were performed to obtain a quadratic model with four independent variables as shown in **Table 1**, and the data on the lipase production by *A. niger* are given in **Table 2**. Because the predicted values obtained from the model fitting technique using the software Design-Expert version 7.0.3 were found to correlate with the observed values, the quadratic polynomial model was seen to be highly significant to represent the actual relationship between the response (lipase activity) and the significant variables. The varied nature of the lipase activities from 3.75 to 26.78 U/mL indicated that the interactions among the factors played a more significant role than the effect of individual factors alone.

The ANOVA for the quadratic regression model (**Table 3**) showed that the model was highly significant, with an *F* value of

Table 3. ANOVA for Quadratic Model for Production of Lipase[a]

source	sum of squares	degrees of freedom	mean square	*F* value	prob (*P*) > *F*
block	65.87	2.00	32.94		
model	892.81	14.00	63.77	62.34	0.0001[b]
A, CSL	534.47	1.00	534.47	522.48	0.0001[b]
B, $NH_4H_2PO_4$	10.34	1.00	10.34	10.11	0.0072[b]
C, sesame oil	55.83	1.00	55.83	54.58	0.0001[b]
D, Na_2HPO_4	1.03	1.00	1.03	1.01	0.3338
AB	29.00	1.00	29.00	28.35	0.0001[b]
AC	53.14	1.00	53.14	51.95	0.0001[b]
AD	10.96	1.00	10.96	10.71	0.0061[b]
BC	12.50	1.00	12.50	12.22	0.0040[b]
BD	16.28	1.00	16.28	15.92	0.0015[b]
CD	0.88	1.00	0.88	0.86	0.3696
A^2	8.12	1.00	8.12	7.94	0.0145
B^2	35.55	1.00	35.55	34.75	<0.0001
residual	13.30	13.00	1.02		
lack of fit	12.31	10.00	1.23	3.73	0.1531
pure error	0.99	3.00	0.33		
corrected total	971.98	29.00			

[a] R^2 = 0.9853; CV = 6.93%. [b] Significant at prob >*F* <0.05.

62.34, as is evident from Fisher's *F* test along with a very low probability value ($P_{model} > F = 0.0001$). At the same time, a relatively lower coefficient of variation (CV = 6.93%) indicated a better precision and reliability of the experiments carried out (*31*). The determination coefficient (R^2) of the model was 0.9853, explaining 98.5% of the variability in the response could be accounted for by the model. Analysis of the design showed a high degree of fitting between predicted and experimental data, which indicated that the model suitably represented the real relationship among the selected factors. The insignificant lack of fit test also indicated that the model suitably represented the experimental data, and the final predictive equation was as follows:

$$\text{lipase activity (U/mL)} = 12.49 + 5.17(A) + 0.72(B) + 1.67(C) - 0.23(D) - 1.35(A)(B) - 1.82(A)(C) - 0.83(A)(D) - 0.88(B)(C) + 1.01(B)(D) + 0.23(C)(D) + 0.95(A^2) - 1.99(B^2) + 3.74(C^2) + 0.46(D^2)$$

It was clear that the three linear coefficients and five quadratic coefficients were highly significant ($P < 0.05$) from the model, and among the four variables, CSL, sesame oil, and $NH_4H_2PO_4$ were the most significant for lipase production, whereas Na_2HPO_4 had a less significant effect on production of lipase. The high *F* value of 522.48, in addition to a least probability value (< 0.0001) for CSL, indicated that CSL played a very prominent role in lipase production, when compared to other variables. A significant quadratic regression, an insignificant lack of fit, and a small total variation (1.47%), which was not explained by the model, suggested that the model precisely represented the data in the experimental region.

The cumulative effect of sesame oil and CSL concentration on lipase production in a medium containing 0.125% $NH_4H_2PO_4$ and 1% Na_2HPO_4 is shown in the response surface plot of **Figure 1**. The lipase activity was 23 U/mL, when the production medium contained 2.0% (w/v) CSL and 2.0% (w/v) sesame oil, whereas decreasing the concentrations of CSL and sesame oil drastically reduced the lipase production levels, confirming that CSL and sesame oil had significant influence on lipase production. Similar results were reported for the production of lipase from *F. oxysporum* (*29*), *Penicillium aurantiogriseum* (*9*), *Rhizopus*

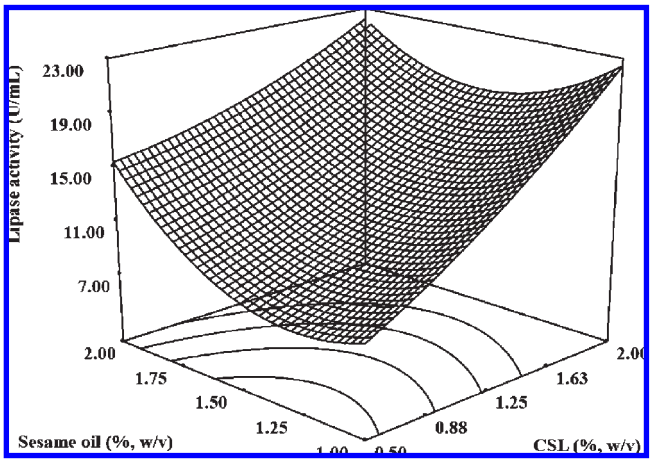

Figure 1. Response surface plot showing the interactive effect of sesame oil and CSL concentration on production of lipase by *A. niger*. Other variables were constant: $NH_4H_2PO_4$, 0.125%; and Na_2HPO_4, 1.0%.

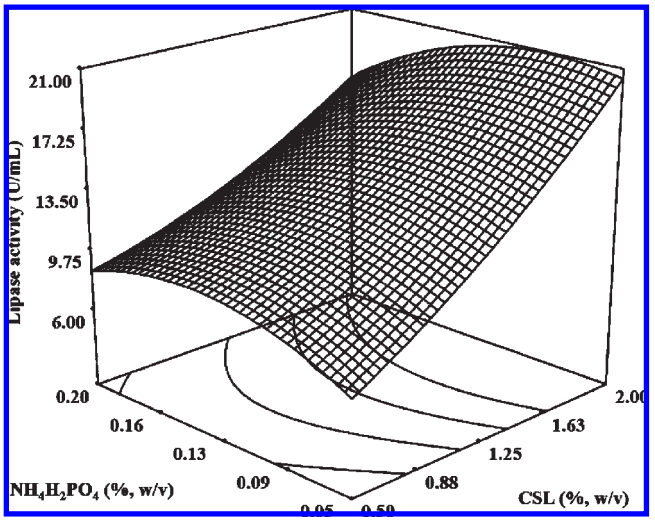

Figure 2. Response surface plot showing the effect of $NH_4H_2PO_4$ and CSL concentration and their mutual effect on lipase production by *A. niger* at 1.0% Na_2HPO_4 1.5% and sesame oil concentrations.

oryzae (*6*, *10*), and *Humicola lanuginosa* (*11*) using CSL as an alternative nitrogen source. Sesame oil was found to be an effective inducer for lipase production by *A. niger*, with an activity of 25 U/mL, which was higher than the activities of *Fusarium solani* (0.88 U/mL) (*32*) and *Candida rugosa* (0.5 U/mL) (*33*). Moreover, this is the first study showing the highest lipase activity using sesame oil as an inducer along with CSL as a nutrition adjunct, because both are renewable agricultural products and economically viable for lipase production at an industrial scale.

The interactive effect of $NH_4H_2PO_4$ and CSL on the production of lipase is shown in **Figure 2**. Maximum lipase activity was obtained with 0.05% $NH_4H_2PO_4$ and 2% CSL concentrations. Further increasing concentations of $NH_4H_2PO_4$ led to decreases in the production of lipase. Tan et al. (*34*) found similar results with *Penicillium camembertii* in a medium supplemented with 0.1% $NH_4H_2PO_4$ for lipase production, whereas the addition of $NH_4H_2PO_4$ completely inhibited the production of lipase in *Penicillium chrysogenum* (*35*). The cumulative effect of Na_2HPO_4 and CSL (**Figure 3**) showed that the lipase activity was optimum when the media contained moderate levels of Na_2HPO_4 (0.75%) with 2% CSL. **Figure 4** depicts the interactive effect of Na_2HPO_4

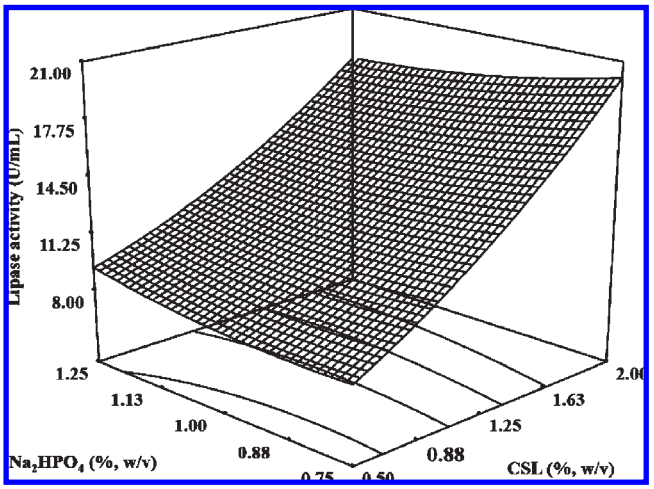

Figure 3. Response surface plot showing the interactive effect of Na_2HPO_4 and CSL on lipase production by *A. niger* in a medium containing 0.125% $NH_4H_2PO_4$ and 1.5% sesame oil.

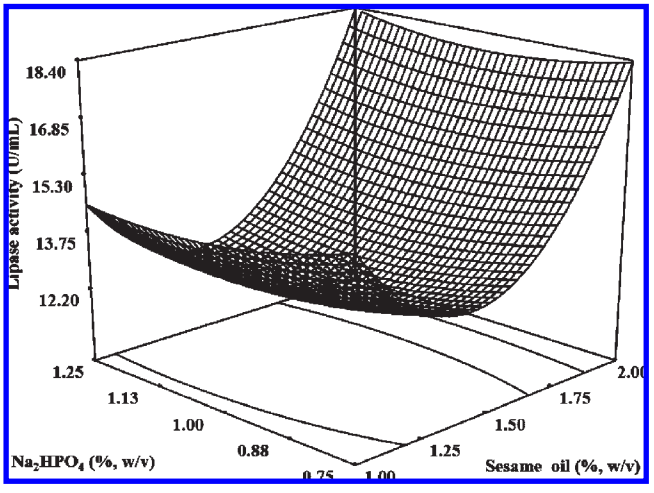

Figure 4. Response surface plot for lipase production showing the interactive effect of Na_2HPO_4 and sesame oil with other variables at 0.125% $NH_4H_2PO_4$ and 1.25% CSL.

and sesame oil with a maximum lipase activity at 0.75% Na_2HPO_4 and 2% sesame oil concentrations. The lipase activity increased with increasing concentrations of sesame oil from 1 to 2%, whereas the production of lipase was inhibited when the media contained a higher concentration of Na_2HPO_4, 1.25%. The maximal lipase activity (26.7 U/mL) obtained under the optimized conditions (**Table 2**) was not depicted in the response surface plots. This could be due to the fact that response surface plots were drawn by imposing constant values (i.e., the central points of the interval taken into consideration) to two of the independent variables of the factorial design. The design illustrated that the optimum values of the factors influencing the lipase production were 2.0% CSL, 0.05% $NH_4H_2PO_4$, 0.75% Na_2HPO_4, and 2.0% sesame oil, with an activity of 26.7 U/mL at 48 h and 30 °C. The lipase produced from *A. niger* was also evaluated for hydrolysis of various vegetable oils and fish oils.

Hydrolysis of Vegetable Oils and Fish Oils. The partially purified lipase of *A. niger* hydrolyzed the vegetable oils and fish oils efficiently, and the hydrolysis ratio reached >60% at 30 °C and 48 h (**Table 4**) except for castor oil, for which the hydrolysis ratio was 54.48%. Among the vegetable oils, palm oil was hydrolyzed efficiently to 71.26% by *A. niger* lipase and is found

Table 4. Effect of Lipase on the Hydrolysis of Vegetable Oils and Fish Oils

	hydrolysis ratio (%)		
oil	24 h	48 h	72 h
sunflower	38.32	60.57	62.82
palm	46.84	71.26	71.84
sesame	42.31	68.13	69.92
olive	39.74	66.57	69.54
castor	40.58	54.48	56.82
sardine	38.21	62.54	64.96
cod liver	36.71	60.19	63.52

to be the second most traded vegetable oil crop in the world, after soy, and has the higher yield of around 5000 kg/ha, when compared to other vegetable oil seed production (*36*). Hence, it is economical to consider palm oil as the feedstock in oleochemical industries for the production of fatty acids and their corresponding esters using enzymes, because they make the process much cleaner and more energy efficient than the conventional thermal fat splitting process, which requires operation at elevated temperature and pressure (*36*). Optimization studies on the hydrolysis of palm oil showed an increase in the hydrolysis ratio of 17.47% at 48 h, when the reaction mixture contained 1 g of oil, 20 mL of 0.1 M phosphate buffer (pH 7.0), and 100 U of lipase. The results on GC analysis of the hydrolyzed palm oil showed the presence of major fatty acids, namely, palmitic acid (43%), oleic acid (37%), and linoleic acid (7%), which are mainly involved in the production of methyl esters as reported by Tan et al. (*37*). *A. niger* lipase also hydrolyzed sardine oil and cod liver oil effectively to ratios of 62.54 and 60.19%, respectively, at 48 h (**Table 4**). Fish oils are rich in n-3 series of polyunsaturated fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have beneficial therapeutic, physiological, and nutritional effects on human health (*38*, *39*). Moreover, the reported hydrolytic ratios are comparatively higher with our lipase, when compared to hydrolytic ratios of 38% (salmon oil), 52.81% (sardine oil) 55.5% (tuna oil), and 60% (cod liver oil) with lipases from *Aspergillus oryzae* (*40*), *Pseudomonas fluorescens* (*41*), *C. rugosa* (*42*), and *Geotrichum candidum* (*43*), respectively. Hence, the lipase from *A. niger* could be effectively used for the hydrolysis of vegetable and fish oils, and studies are in progress to show the application of this enzyme in methanolysis of vegetable oils and in targeted recovery of polyunsaturated fatty acids.

Conclusion. Having established that *A. niger* lipase could be effectively used as an additive in detergent formulation, it is of utmost relevance to produce the enzyme in an economically feasible medium. CSL, a cost-effective nutrition adjunct as well as an agro-industrial residue, was found to be the suitable complex nitrogen source, which could replace the meat extract in the medium without significant difference in the production of enzyme with sesame oil as an inducer. The results of the RSM clearly showed that the influential parameters for the lipase production were 2.0% CSL, 0.05% $NH_4H_2PO_4$, 0.75% Na_2HPO_4, and 2.0% sesame oil, with an activity of 26.7 U/mL at 48 h and 30 °C, which was 2.16-fold higher than the activity obtained by the conventional one-factor-at-a-time method. The utilization of CSL and sesame oil in the medium led to a reduction in the overall cost of lipase production medium by 55−60% of the total medium cost, thereby making the process economically attractive and industrially feasible. Furthermore, the lipase could also be utilized in the hydrolysis of vegetable oils and fish oils, which are economically and industrially important for the production of fatty acid esters and n-3 series of polyunsaturated fatty acids.

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