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Optimization of processing parameters for extraction of total, insoluble and soluble dietary fibers of defatted rice bran

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Abstract

Response surface methodology was used to optimize the processing parameters for extraction of total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) of defatted rice bran (DRB). The studied independent factors were concentration of NaOH solution (varying from 0.15 to 0.25 mol/L), soaking time (from 60 to 90 min), α-amylase enzyme – substrate (E: S) ratio (from 0.6:100 to 0.9:100 g: g of dry DRB) and alcalase enzyme concentration (from 3.5:100 to 4.7 g: g of dry DRB)) whereas; the dependent variables were extraction yield and purity of TDF, IDF and SDF. Therefore, the three- level four-factor Box-Behnken design was used to establish the optimum conditions and the generated regression quadratic polynomial models and adequacy of each dependent variable were significant ($p < 0.0001$) with regression coefficient R2 (> 0.90) and lack of-fit was not significant. Moreover, ANOVA showed that most of the linear, interaction and quadratic regression coefficient values were significant ($p < 0.05$). The optimum processing parameters observed for extraction of TDF, IDF and SDF with high yield and purity were: 0.15 mol/L NaOH solution concentration, 64.3 min soaking time, 0.68:100 and 3.52:100 (g: g) α-amylase and alcalase enzyme – substrate ratio (E: S), respectively. Moreover, the alkali pretreatment was the factor amongst the others that significantly ($p<0.05$) affected the purity of Fiber fractions but did not contribute to improve their yields.

Key words: Dietary fibers, Optimization, Purity, Response surface methodology, Yield

Introduction

The measurement of dietary fibers in foods is complexes issue not only the choice of analytical method, but also the definition of fiber. The gravimetric techniques, which were earliest and included crude fiber, acid detergent fiber, and neutral detergent fiber, grossly underestimate dietary fiber content and are being replaced by new and more accurate methods (Dreher, 1987). Therefore, total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) from food or plant material are determined using AOAC enzymatic-gravimetric method (Prosky et al., 1988).

Different conditions for extracting TDF, IDF and SDF from cereal bran were previously studied by Cartaňo and Juliano (1970), Theandor and Westerlund (1986), Aoe et al. (1993) and Thumthanarak (1996). They reported that the yield and the quality of dietary fibers are dependent of the relevant conditions used to extract the fibers. Numerous other studies have also shown that the extraction of non-starch polysaccharides was significantly affected by the extraction conditions (Wu et al., 2007; Yin and Dang, 2008; Liu et al., 2009; Li et al., 2009; Qiao et al., 2009; Zhao et al., 2009; Guo et al., 2010; Sun et al., 2010; Zhu et al., 2010).

It is evident that the optimum TDF, IDF and SDF yields cannot be estimated based on just the one-factor (single factor approach). But, the interaction between extraction conditions may be included in the determination of the yield and quality. A study is needed not only to determine the optimum extraction conditions to obtain a desirable yield or quality of TDF, IDF and SDF from defatted rice bran but is also required to understand

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the degree of interactions between the extraction conditions such as concentration of enzymes, soaking time in the solvent (NaOH) and solvent concentration. A statistical optimization procedure such as response surface methodology (RSM) includes the interaction of the extraction factors into computation (Haaland, 1989).

RSM is widely applied in the food industry to determine the effects of multiple processing variables and their interaction on response variables (Vasquez and Martin, 1998; Senanayake and Shahidi, 2002; Tellez-Luis et al., 2003). Myers and Montogomery (2002) have expressed that RSM can be used to develop, improve, and optimize a process because the methodology includes both statistical and mathematical techniques. The RSM reduces the number of experimental trials and is considered to be less laborious and time consuming to optimize a process (Glovanni, 1983). The RSM can also be applied to an experimental design such as Box-Behnken (BBD) with three factors and three level variables to fit a second-order polynomial by least squares (Liu et al., 2009).

The objectives of this study were to a) determine the optimal composition of DRB and DRB fiber content b) determine effects of single factors such as NaOH concentration, soaking time in the NaOH solution, concentrations of α-amylase and alcalase to DRB on TDF, IDF and SDF yield and purity, and c) optimize the processing parameters such as NaOH concentration, soaking time, concentrations of α-amylase and alcalase on the TDF, IDF and SDF yield and purity using RSM with a three level and four independent variables Box-Behnken factorial design.

Materials and Methods

Proximate analysis of defatted rice bran

Moisture, protein, fat and ash contents of DRB were determined according to AACC approved methods 44-15A, 4-13, 30-25 and 08-17 respectively (AACC 2000). TDF, IDF and SDF contents of DRB were determined according to AACC method 32-07 using an enzymatic-gravimetric method with phosphate buffer (0.08 M, pH 6.0).

Extraction of TDF, IDF and SDF from defatted rice bran

TDF, IDF and SDF were extracted (Figure 1) by a modified procedure of Mirko (Mirko et al., 2003). DRB (10 g) was pretreated with NaOH solution. DRB was soaked with NaOH solution at different concentrations during different times at room temperature. After chemical pretreatment DRB was separated by centrifugation and washed with distilled water to neutral pH and then TDF, IDF, and SDF were extracted by enzymatic - gravimetric procedure of Mirko et al. (2003).

For enzymatic hydrolysis two (2) types of α-amylase: termamyl α-amylase 20.000 U/g and α-amylase 3000 U/g (Wuxi Enzyme Factory), and four (4) types of protease: neutrase 0.8L, alcalase 2.4 L (Novozymes Biological Engineering Beijing), flavourzyme 500 LAPU/g, protamex 1.5 AU/g (Wuxi Enzyme Factory) were used to select the best one. Termamyl α-amylase and Alcalase were selected according their degree of hydrolyze (DH) (Unpublished data). Enzymatic hydrolysis was completed by incubation with amyloglucosidase (100000 U/g Novozymes Biological Engineering).

Optimization of extraction of TDF, IDF, and SDF from DRB

The best combinations of the variable factors for the extraction of TDF, IDF, and SDF were determined using a three levels and four independent variables Box-Behnken factorial Design (BBD) (Sun et al., 2009). The independent extraction variables including concentration of NaOH solution (X_1), soaking time (X_2), and α-amylase – dry DRB ratio (X_3), and alcalase – dry DRB ratio (X_4) were used for this study.

The optimum range of X_1, X_2, X_3, and X_4 were determined based on the single factor experiment for extraction of TDF, IDF, and SDF. The variables (X_1, X_2, X_3, and X_4) were coded according to Eq. 1) for statistical calculation:

$$i = (Xi - Xo) / \Delta X \quad \text{(Eq.1);}$$

Where i was the coded value of the variable; Xi was the actual value of the independent variable Xo was the actual value of the independent variable at the center point, and ΔX was the step change value of the independent variable.

Table 1. Independent variable and levels used in the Response Surface Design.

Independents variables	Factor levels		
	-1	0	1
X_1: Concentration of NaOH (mol/L	0.15	0.2	0.25
X_2: Soaking time (min)	30	60	90
X_3: α-amylase –dry DRB (E:S) ratio (g:g)	0.6	0.75	0.9
X_4: alcalase –dry DRB (E:S) ratio (g:g)	3.5	4.1	4.7

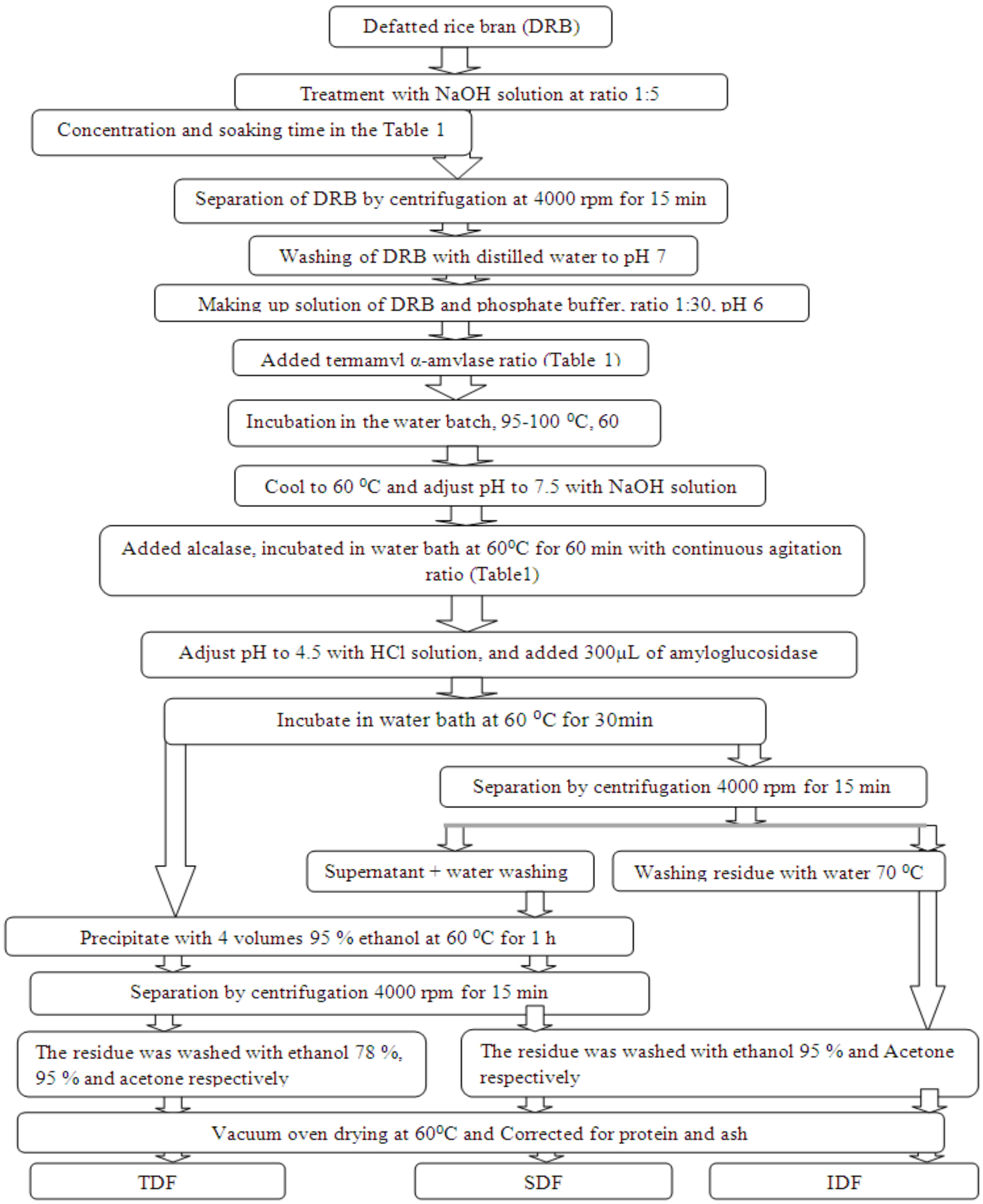

Figure 1. TDF, IDF, and SDF from DRB extraction flow diagram.

The dependents variables were the yields (%, w/w) and the purity (%) of TDF, IDF, and SDF from DRB in this experimental design. The complete experimental design consisted of 29 experimental points. All of the 29 treatments were taken in random order. The conditions at the center of BBD were repeated five times (2, 5, 6, 11, and 26) to estimate the pure error sum of squares (Zhu et al., 2010).

Data analysis

Mean values from the repeated or separated analyses were reported with standard deviation. The statistical significance of observed differences among treatment means was evaluated by analysis of variance (ANOVA).

Data from BBD were analyzed by multiple linear regressions to fit the quadratic polynomial model equation (2).

$$Y = \beta 0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} x_i x_j \quad \text{(Eq. 2)}$$

Where, Y is the dependent variables (yield or purity of TDF, IDF, and SDF from DRB); β_0, β , β , and β are the coefficient regression of constant, linear, quadratic and interactive terms, respectively; and are the coded independent variables.

The regression coefficients of linear, quadratic and interaction terms were determined based on ANOVA and the P-value (0.05) of each coefficient was considered to be statistically significant. The 3-D response surface plots and contour plot were obtained using the response value along with two independent variables, while the other variables were the fixed constants at their respective 0 level (center value of the testing range).

Results and Discussion

Optimal composition of DRB

The Table 2 shows the proximate composition of defatted rice bran. The total dietary fiber (TDF) content of DRB is 32.98% (w/w) while, its IDF and SDF contents are 30.2% and 2.7% (w/w) respectively. These values of TDF and IDF contents were higher than those found by Abdul-Hamid while the values of SDF content were similar (Abdul-Hamid, 2000).These results showed that IDF represents the major component (93.84 %) while SDF content is only 6.16% (w/w) of rice bran dietary fiber.

Table 2. Chemical composition of defatted rice bran (DRB).

Proximate composition of DRB (% of dry weight of DRB)	
Moisture	8.7±0.03
Protein	16.2±0.2
Fat	2.8±0.05
Ash	10.7±0.01
TDF	32.9±0.3
IDF	30.2±0.4
SDF	2.7±0.15

Values are means ± standard deviation of 3 replicates (n=3) TDF =Total dietary fiber, IDF = Insoluble dietary fiber and SDF = Soluble dietary fiber

Effect of single factor condition on the yield and purity of TDF, IDF and SDF of DRB

The result of the extraction yield and the purity of TDF, IDF and SDF according to the Box-Behnken design matrix of four variables are shown in Table 3.The highest yield values were 31.11, 26.88 and 2.69 % under the extraction conditions 9, 13 and 7 for TDF, IDF and SDF, respectively. While, the highest purity values were 81.84, 90.00 and 53.57% under conditions 25 for TDF, IDF and 19 for SDF.

Effect of single factor on yield and purity of TDF

The yield of TDF ranged from 26.05 to 31.11%, while the purity ranged from 75.97 to 81.84%. The extraction yield increased with the decrease of NaOH concentration (Figure 2A) while increasing soaking time had moderate effect on it (Figure 2B). Jackson (1977) reported that alkali pretreatment is required to alter the structure of cellulosic biomass than dispirits the cell wall by dissolving hemicellulose and lignin, by hydrolyzing uronic and acetic acid esters and by swelling cellulose and decreasing the crystallinity of cellulose. Melinda et al. (2007) have confirmed in their study "Corn fiber as a raw material for hemicellulose and ethanol production". Thus, pretreatment with high concentration of alkali solution might cause some loss of polysaccharide which can explain the decrease of yield of TDF with increasing NaOH solution concentration.

Inversely the purity of TDF has been increased with the increase of NaOH concentration from 78.39 to 81.04% (Figure 2A). The purity of TDF have been increased during the first 60 min of soaking after that it is moderately decreased (Figure 2B). The alteration of the cell wall of lignocellulosic substances by alkali solution makes them more accessible to the enzymes (Melinda et al., 2007) which might improve the hydrolysis of starch and protein and then increased the purity of fiber.

The increase of Termamyl – DRB ratio from 0:100 to 0.75:100 increased the purity of TDF but, its yield was not significantly affected (Figure 2C). Alcalase - DRB ratio also affected the TDF purity. The yield was not affected with an increasing of alcalase – DRB ratio while, the purity was increased with an increase of E: S ratio from 3.5:100 to 4.1:100 and after that it decreased (Figure 2D).

Effect of single factor on yield and purity of IDF

Similar to TDF, the yield of IDF also increased at lower concentrations of NaOH (Figure 2A). But, the soaking time and enzymes: DRB ratio did not significantly affect the yield (Figure 2B, 2C and 2D). The purity of IDF was affected by the single factor in the same way as the purity of TDF (Figure 2A, 2B, 2C and 2D). The changes in IDF yield and purity due to the effects of single factors might have similar explanation with TDF.

Effect of single factor on yield and purity of SDF

The yield of SDF was moderately or not affected by all single factors (NaOH concentration, soaking time, and α-amylase and alcalase – DRB ratio) (Figure 2A, 2B, 2C and 2D). However, the purity of SDF increased with the increase of NaOH concentration and soaking time (Figure 2A and 2B). On other hand, the purity of SDF was higher when amylase – dry DRB ratio and alcalase – dry DRB ratio were 0.6:100 and 3.5:100 respectively. After that the purity decreased (Figure 2C and 2D).

Contrary to what has been reported by a number of authors (Cartaňo and Juliano, 1970; Aoe et al., 1993; Yuting, 2008; Teramoto et al., 2008; Buranov and Mazza, 2010) that the chemical extraction (alkaline or acid) of SDF increases their yield, the alkaline pretreatment of DRB could not increase the yield of SDF. However, the pretreatment favorably improved their purity. The reasons are probably the same previously cited above for TDF and DTF.

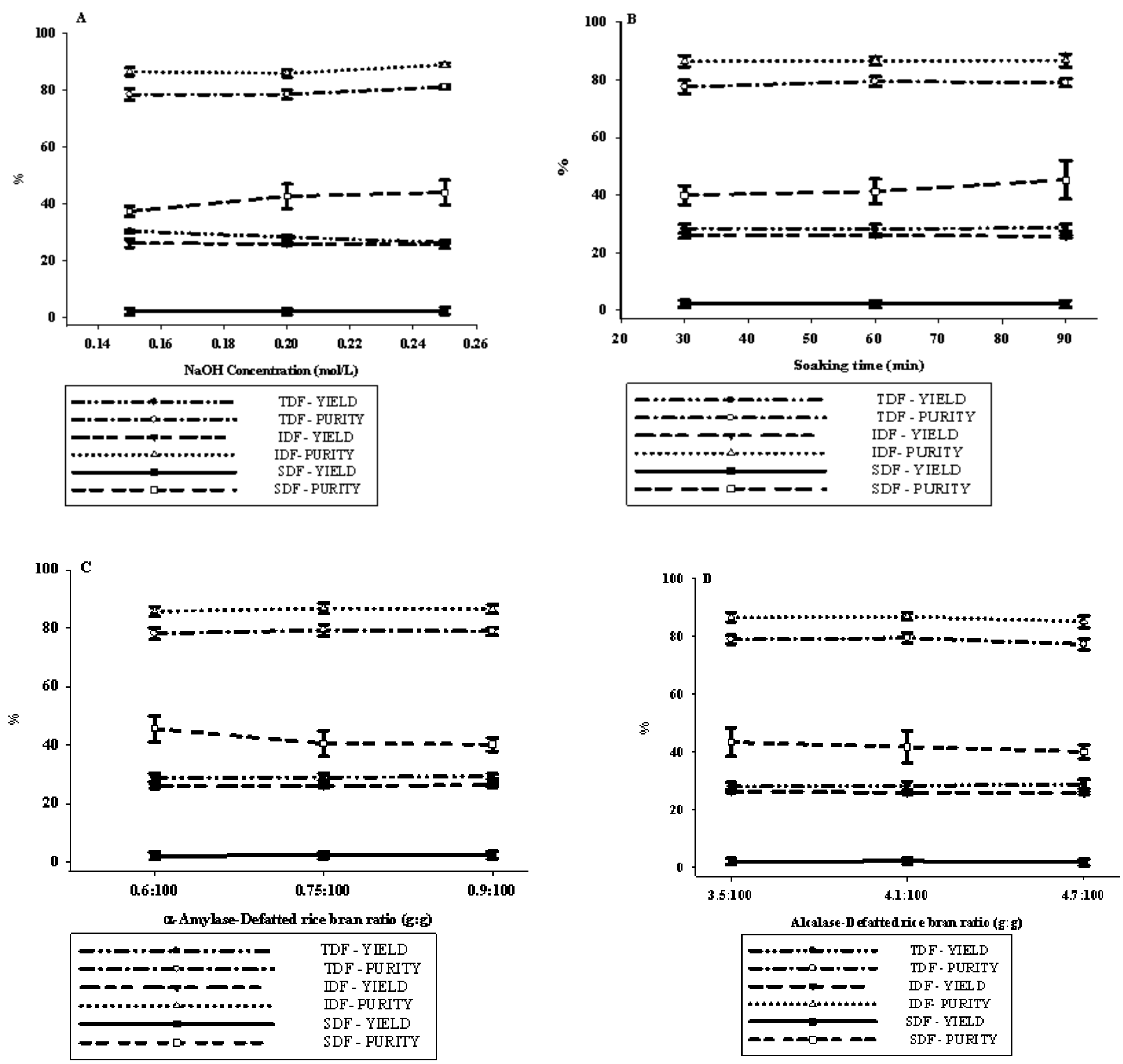

Figure 2. Effects of NaOH concentration (A) , soaking time in the NaOH solution (B), α-Amylase- Defatted rice bran ratio (C) and Alcalase- Defatted rice bran ratio (D) on the Yield and Purity of TDF, IDF and SDF from defatted rice bran.

Optimization of extraction conditions for TDF, IDF, and SDF from DRB

The extraction conditions were optimized by a second order polynomial equation using a 29-run BBD with four factors and three levels including five replicates at the center point. The experimental conditions according to the factorial design in coded units with the uncoded experimental values are shown in Table 3.

Optimization of extraction yield and purity of TDF

Maximum yield (Y1) and purity (Y2) of TDF were obtained under the extraction conditions of treatments 9 and 25-26 (Table 3), respectively. By analyzing the experimental data through multiple regressions, second-order polynomial equations were generated in terms of coded values as follow:

Yield: $Y1= 27.93 - 2.04X_1 + 0.14X_2 - 0.24X_3 + 0.39X_4 + 0.29X_1X_2 + 0.001256X_1X_3 – 0.30X_1X_4 – 0.36X_2X_3 – 0.12X_2X_4 + 1.22X_3X_4 + 0.20X_1^2 + 0.41X_2^2 + 0.22X_3^2 + 0.21X_4^2$

Purity: $Y2= 80.32 + 1.33X_1 + 0.73X_2 + 0.36X_3 - 0.81X_4 – 1.50 X_1X_2 - 0.35X_1X_3 + 1.37X_1X_4 - 0.71X_2X_3 – 0.61 X_2X_4 + 0.25X_3X_4 + 0.76 X_1^2- 1.46X_2^2- 1.06X_3^2- 1.58X_4^2$

The ANOVA of fitted quadratic polynomial model of extraction showed that the model was significant (p<0.05, n=3 and lack of fit was insignificant (Table 4).

Table 3. Box-Behnken Design Matrix of four variables.

Treatments	X_1	X_2	X_3	X_4	Yield TDF	Purity TDF	Yield IDF	Purity IDF	Yield SDF	Purity SDF
					Y_1 (%)	Y_2 (%)	Y_3 (%)	Y_4 (%)	Y_5 (%)	Y_6 (%)
1	0	-1	0	-1	27.91	76.62	26.73	84.4	2.19	41.05
2	0	0	0	0	27.91	80.36	25.7	86.45	2.31	38.75
3	0	0	-1	1	27.75	76.23	25.46	83.88	1.66	38.45
4	1	0	0	-1	26.18	80.15	26.01	88.24	2.42	47.01
5	0	0	0	0	27.97	80.03	25.98	86.45	2.32	38.81
6	0	0	0	0	27.97	80.36	25.52	86.58	2.31	36.64
7	1	0	1	0	26.05	81.27	26.32	88.92	2.69	41.84
8	-1	-1	0	0	30.74	75.97	26.46	85.84	2.29	37.23
9	-1	0	0	1	31.11	76.04	26.16	84.34	1.94	37.91
10	-1	1	0	0	30.4	80.35	25.37	87.95	2.4	34.41
11	0	0	0	0	27.9	80.36	25.93	86.45	2.24	41.88
12	1	1	0	0	26.91	80.23	25.53	88.66	2.35	50.4
13	0	-1	1	0	28.54	78.34	26.88	85.58	2.14	44.34
14	0	0	-1	-1	29.41	78.23	26.58	85.29	2.07	49.38
15	-1	0	-1	0	30.65	78.18	26.51	85.7	2.16	39.47
16	0	1	1	0	28.13	78.15	25.03	87.1	2.43	44.36
17	0	0	1	1	29.74	77.59	26.59	84.55	2.03	43.49
18	1	0	0	1	26.4	81.14	25.93	88.52	2.23	38.89
19	0	1	-1	0	29.32	78.62	26.33	85	1.86	53.57
20	-1	0	0	-1	29.7	80.54	26.72	87.62	2.16	36.93
21	0	0	1	-1	26.53	78.61	26.57	86.24	2.19	39.49
22	0	-1	0	1	28.89	76.26	25.35	86.45	2.1	39.91
23	0	-1	-1	0	28.3	75.98	24.79	86.05	2.39	41.77
24	-1	0	1	0	30.12	79.23	25.95	87.05	2.26	38.23
25	1	-1	0	0	26.1	81.84	25.38	90	2.66	35.29
26	0	0	0	0	27.9	80.49	25.82	86.45	2.28	44.18
27	1	0	-1	0	26.57	81.61	25.39	88.54	2.15	50.56
28	0	1	0	-1	28.43	79.61	25.82	88.37	2.145	46.52
29	0	1	0	1	28.92	76.83	25.82	83.08	1.91	42.44

Table 4. ANOVA of fitted quadratic polynomial model of extraction Yield (Y1) and Purity (Y2) of TDF from DRB.

Source	DF		SS		MS		F value		Prob. > F	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
Model*	14	14	61.31	97.42	4.38	6.96	3660.14	139.98	<0.0001	<0.0001
Residual	14	14	0.017	0.70	0.001197	0.050	—		—	
Lack of fit**	10	10	0.011	0.58	0.001126	0.058	0.82	1.96	0.6381	0.2695
Pure Error	4	4	0.005494	0.12	0.001373	0.029	—		—	
Total	28	28	61.33	98.12	—		—		—	

DF=Degree of Freedom; SS= Sum of Squares; MS= Mean Square, Model*= Model significant, and Lack of fit**= lack of fit not significant.

The analysis of variance, goodness-of-fit and the adequacy of the models showed that the models were found to be adequate for prediction with the range of selected experimental variables. The means were 28.36±0.035 and 78.94±0.22 for yield and purity, respectively. The determination coefficients (R^2= 0.9997 for yield and R^2= 0.9988 for purity) obtained by ANOVA of the quadratic regression models indicated that only 0.03 % and 0.02 % of the total variation were not explained by the models for yield and purity, respectively. The value of adjusted determination coefficients (Adjusted R^2 = 0.9995 and Adjusted R^2 = 0.9858 for yield and purity, respectively) indicated a high degree of correlation between the observed values, while a lower coefficient of variation values of yield (0.12) and purity (0.28) showed the experimental values were reliable (Liyana-Pathirana and Shahidi, 2005).

The regression parameters of the predicted response surface quadratic models for yield and purity of TDF from DRB was shown in Table 4 The results indicated that only the interaction between NaOH concentration and α-amylase –DRB ratio (X_1X_3) was not significant (p>0.05, n=3) for the extraction yield, while all linear, quadratic and interaction between factors were significant (p<0.05, n=3) for the purity of TDF. The P-value was used to verify the significance of each coefficient and to describe the degree of interactions strength between the variables (Muralidhar et al., 2001). A smaller P-value means the corresponding coefficient is more significant (Muralidhar et al., 2001).

The result of statistical analysis showed that the entire factor (NaOH concentration, soaking time, amylase and alcalase DRB ratio) significantly (P <0.05, n=3 for both models) affected the yield and purity of TDF (Table 5).

The interaction regression coefficients X_1X_3 of yield quadratic polynomial model was not significant, which indicated the interaction between NaOH solution and α-amylase – DRB ratio did not affected the yield of TDF. While, all the interaction regression coefficients of purity polynomial model were significant thus, the interactions between all independent factors (NaOH concentration, soaking time α-amylase and alcalase DRB ratio affected the purity of TDF.

Table 5. Regressions Coefficients of the Predicted Quadratic Polynomial Models.

Parameters	Coefficient Estimate		Standard Error		P-value*	
	Yield	Purity	Yield	Purity	Yield	Purity
X_1	-2.04	1.33	0.009986	0.18	< 0.0001	< 0.0001
X_2	0.14	0.73	0.009986	0.18	< 0.0001	< 0.0001
X_3	-0.24	0.36	0.009986	0.18	< 0.0001	< 0.0001
X_4	0.39	-0.81	0.009986	0.18	< 0.0001	< 0.0001
X_1X_2	0.29	-1.50	0.017	0.31	< 0.0001	< 0.0001
X_1X_3	0.001256	-0.35	0.017	0.31	0.0943	0.0076
X_1X_4	-0.30	1.37	0.017	0.31	< 0.0001	< 0.0001
X_2X_3	-0.36	-0.71	0.017	0.31	< 0.0001	< 0.0001
X_2X_4	-0.12	-0.61	0.017	0.31	< 0.0001	< 0.0001
X_3X_4	1.22	0.25	0.017	0.31	< 0.0001	0.0001

*P-value less than 0.0500 indicate model terms are significant (n=3)

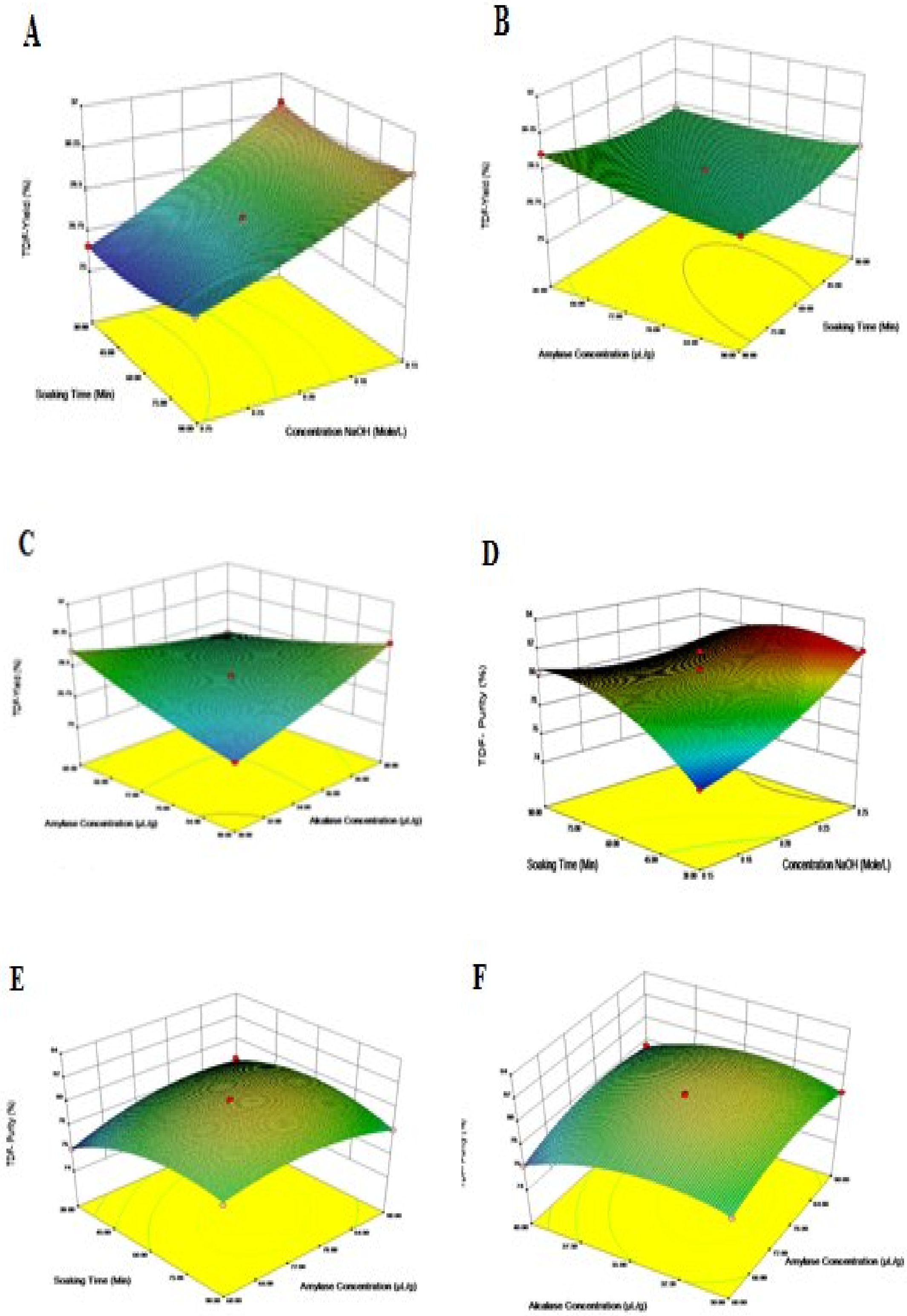

Figure 3. Response Surface Plots (3-D) showing the effects of interaction of some variable factors on yield and purity of TDF from defatted rice bran.

Table 6. ANOVA of fitted quadratic polynomial model of extraction Yield (Y3) and Purity (Y4) of IDF from DRB.

Source	DF		SS		MS		F value		Prob. > F	
	Y_3	Y_4	Y_3	Y_4	Y_3	Y_4	Y_3	Y_4	Y_3	Y_4
Model*	14	14	8.07	78.82	0.58	5.63	24.06	2074.73	<0.0001	<0.0001
Residual	14	14	0.34	0.038	0.024	0.002714	—		—	
Lack of fit**	10	10	0.20	0.024	0020	0.002447	0.59	0.81	0.7725	0.6456
Pure Error	4	4	0.14	0.014	0.034	0.003380	—		—	
Total	28	28	8.40	78.86	—		—		—	

DF=Degree of Freedom; SS= Sum of Squares; MS= Mean Square, Model*= Model significant, and Lack of fit**= lack of fit not significant

The response surface plots (3-D) of yield and purity of TDF from DRB affected by the interaction of NaOH concentration and soaking time, the interaction of soaking time and α-amylase concentration and the interaction between ratios of α-amylase-DRB and alcalase- DRB are shown in the Figure 3.

We fond that an increasing of the NaOH solution concentration and soaking time has led in lower yield and higher purity of TDF (Figure 3 A and D). However the combination of a short soaking time with a low DRB-α-amylase ratio gives a high yield of TDF (Figure 3 B) whereas, the combination of low α-amylase- DRB with alcalase- DRB ratio also leads to a high yield (Figure 3 C). The higher purity was reached when the soaking time and α-amylase-DRB ratio or ratios of α- amylase and alcalase were in the range (Figure 3 E, F).

As mentioned above alkali altered cell wall of lignocellulosic substance (Jackson, 1977) thus, the long soaking time of DRB in the high concentrate NaOH solution might cause the loss of some soluble polysaccharide hence the low yield of TDF. While, the short soaking time of DRB will favorite the access of enzyme to substrate which can improve the yield. Another hand the higher purity was due to the alkali pretreatment which facilitates the access of enzymes to DRB matrix therefore improves the degree of hydrolysis starch and protein.

Optimization of extraction yield and purity of IDF

Maximum yield (Y_3) and purity (Y_4) of IDF were obtained under the extraction conditions of treatments 13 and 25 (Table 3), respectively. The second-order polynomial equations were generated in terms of coded values as follow:

Yield: $Y_3 = 25.79 - 0.22X_1 - 0.14 X_2 + 0.19X_3 - 0.26X_4 + 0.31X_1X_2 + 0.37X_1X_3 + 0.12X_1X_4 - 0.85 X_2X_3 + 0.34 X_2X_4 + 0.28X_3X_4 + 0.084X_1^2 - 0.20X_2^2 + 0.17X_3^2 + 0.34X_4^2$

Purity: $Y4 = 86.48 + 1.20X_1 + 0.15X_2 + 0.42X_3 - 0.78X_4 - 0.86X_1X_2 - 0.24X_1X_3 + 0.89X_1X_4 + 0.64X_2X_3 - 1.84X_2X_4 - 0.070X_3X_4 + 1.63X_1^2 + 0.016X_2^2 - 0.56X_3^2 - 0.92X_4^2$

The ANOVA of fitted quadratic polynomial model of extraction showed that the model was significant ($p<0.05$, n=3) and lack of fit insignificant (Table 6).

The analysis of variance, goodness-of-fit and the adequacy of the models showed that the R-square values indicated that only 3.99% (R^2 = 96.01%) and 0.05 % (R^2 = 99.95%) of the total variation were not explained by the models of extraction yield and purity of IDF, respectively. Similar to TDF the adjusted R-square (92.02% and 99.90% for yield and purity, respectively) also indicated a high degree of correlation between the observed values, while a lower coefficient of variation values of yield (0.60) and purity (0.060) showed the experimental values were reliable (Liyana-Pathirana and Shahidi, 2005).

The regression parameters of the predicted response surface quadratic models for yield and purity of IDF from DRB was shown in Table 7. The results indicated that X_1X_4 and X_1^2 were not highly significant ($p>0.05$ n=3) for extraction yield, while X_2^2 was not significant ($p>0.05$, n=3) for purity of IDF.

According ANOVA the yield and purity of IDF were significantly ($p<0.05$ n=3) affected by all the variables factors except interaction between concentrations of NaOH and alcalase and quadratic NaOH solution concentration ($P>0.05$ n=3).

The response surface plots (3-D) of yield and purity of IDF from DRB affected by the interaction of NaOH concentration and soaking time, the interaction of soaking time and α-amylase concentration and the interaction between ratios of α-amylase-DRB and alcalase-DRB are shown in the Figure 4.

The effects of interactions between NaOH concentration-Soaking time, α-amylase-DRB ratio-Soaking time and α-amylase-DRB ratio-alcalase-DRB ratio on the yield and purity of IDF were similar to TDF.

Table 7. Regressions Coefficients of the Predicted Quadratic Polynomial Models.

Parameters	Coefficient Estimate		Standard Error		P-value	
	Yield	Purity	Yield	Purity	Yield	Purity
X_1	-0.22	1.20	0.045	0.015	0.0003	< 0.0001
X_2	-0.14	0.15	0.045	0.015	0.0073	< 0.0001
X_3	0.19	0.42	0.045	0.015	0.0008	< 0.0001
X_4	-0.26	-0.78	0.045	0.015	< 0.0001	< 0.0001
X_1X_2	0.31	-0.86	0.077	0.026	0.0013	< 0.0001
X_1X_3	0.37	-0.24	0.077	0.026	0.0003	< 0.0001
X_1X_4	0.12	0.89	0.077	0.026	0.1384	< 0.0001
X_2X_3	-0.85	0.64	0.077	0.026	< 0.0001	< 0.0001
X_2X_4	0.34	-1.84	0.077	0.026	0.0005	< 0.0001
X_3X_4	0.28	-0.070	0.077	0.026	0.0025	0.0177

P-value less than 0.0500 indicate model terms are significant (n=3)

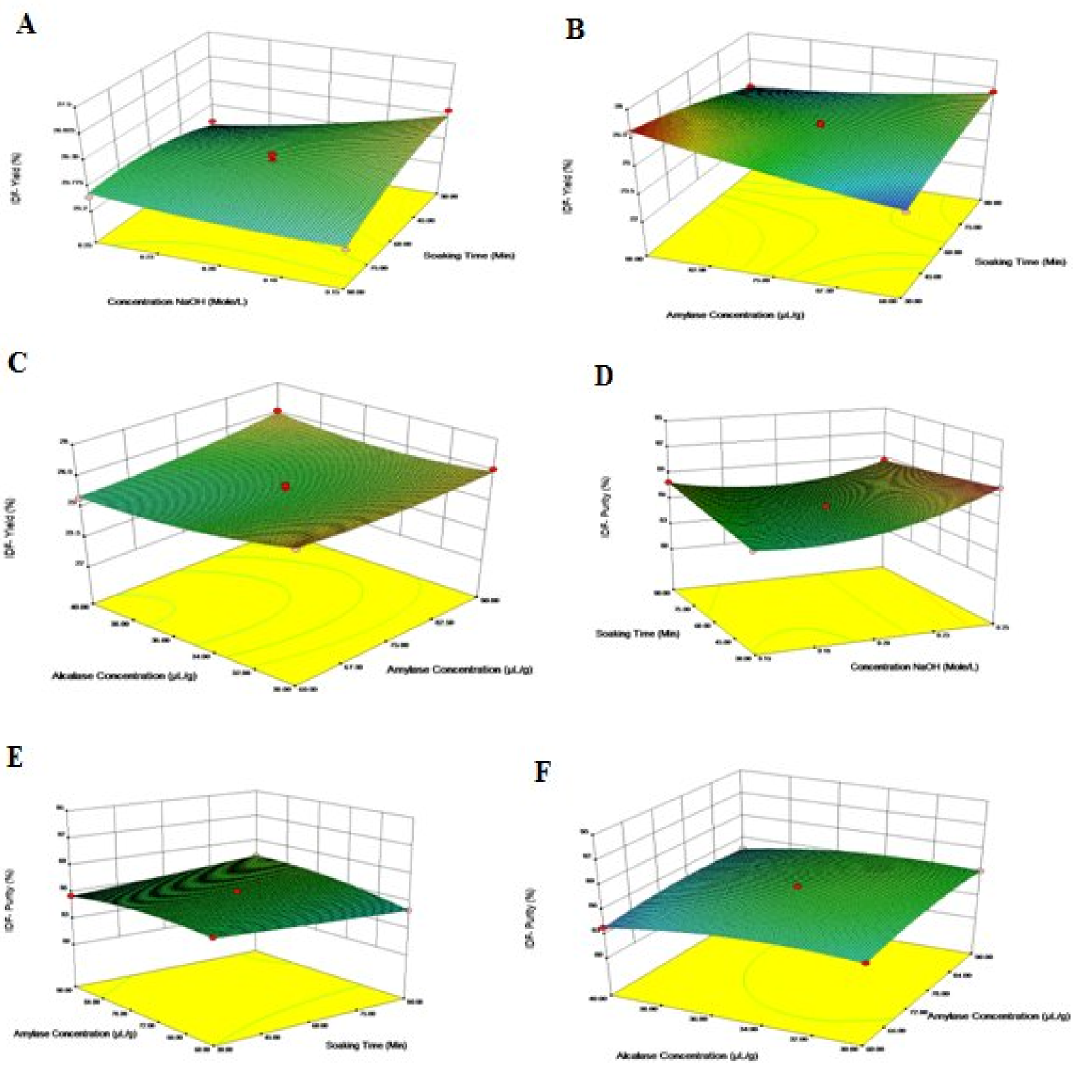

Figure 4. Response Surface plots (3-D) showing effects of interaction of some variable factors on the yield and purity of IDF from defatted rice bran.

Table 8. Analysis of variance of fitted quadratic polynomial model of extraction yield (Y5) and purity (Y6) of SDF from defatted rice bran.

Source	DF		SS		MS		F value		Prob. > F	
	Y_5	Y_6	Y_5	Y_6	Y_5	Y_6	Y_5	Y_6	Y_5	Y_6
Model*	14	14	1.29	604.50	0.092	43.18	41.06	9.72	<0.0001	<0.0001
Residual	14	14	0.031	62.16	0.0022	4.44	—		—	
Lack of fit**	10	10	0.027	26.92	0.0027	2.69	2.73	0.31	0.1730	0.9416
Pure Error	4	4	0.004	35.25	0.0010	8.81	—		—	
Total	28	28	1.32	666.67	—		—		—	

DF=Degree of Freedom; SS= Sum of Squares; MS= Mean Square, Model*= Model significant, and Lack of fit**= lack of fit not significant

Table 9. Regressions Coefficients of the Predicted Quadratic Polynomial Models.

Parameters	Coefficient Estimate		Standard Error		P-value	
	Yield	Purity	Yield	Purity	Yield	Purity
X_1	0.11	3.32	0.014	0.61	< 0.0001	< 0.0001
X_2	-0.056	2.67	0.014	0.61	0.0012	0.0006
X_3	0.12	-1.79	0.014	0.61	< 0.0001	0.0108
X_4	-0.11	-1.61	0.014	0.61	< 0.0001	0.0193
X_1X_2	-0.11	4.48	0.024	1.05	0.0005	0.0008
X_1X_3	0.11	-1.87	0.024	1.05	0.0004	0.0979
X_1X_4	0.008128	-2.28	0.024	1.05	0.7367	0.0486
X_2X_3	0.20	-2.94	0.024	1.05	< 0.0001	0.0143
X_2X_4	-0.037	-0.74	0.024	1.05	0.1369	0.4965
X_3X_4	0.062	3.73	0.024	1.05	0.0195	0.0033

P-value less than 0.0500 indicate model terms are significant (n=3)

Optimization of extraction yield and purity of SDF

Maximum yield (Y_5) and purity (Y_6) of SDF were obtained under the extraction conditions of treatments 7 and 19 (Table 3), respectively. The second-order polynomial equations were generated in terms of coded values as follow:

Yield: $Y_5 = 2.29 + 0.11X_1 - 0.056X_2 + 0.12X_3 - 0.11X_4 - 0.11X_1X_2 + 0.11X1X3 + 0.008128X_1X_4 + 0.20X_2X_3 - 0.037X_2X_4 + 0.062X_3X_4 + 0.12X_1^2 + 0.011X_2^2 - 0.094X_3^2 - 0.22X_4^2$

Purity: $Y_6 = 40.05 + 3.32X_1 + 2.67X_2 – 1.79X_3 – 1.61X_4 + 4.48X_1X_2 – 1.87X_1X_3 – 2.28X_1X_4 – 2.94X_2X_3 – 0.74X_2X_4 + 3.73X_3X_4 – 1.21X_1^2 + 1.68X_2^2 + 3.39X3^2 + 0.45X_4^2$

The ANOVA of fitted quadratic polynomial model of extraction showed that the model was significant ($p<0.05$ n=3) and lack of fit insignificant (Table 8).

The analysis of variance, goodness-of-fit and the adequacy of the models showed the R-square values indicated that only 2.38% and 9.32% of the total variation were not explained by the models of extraction yield and purity of SDF, respectively while, the adjusted R-square 95.25% and 81.35% also indicated high degree of correlation between values. The regression parameters of the predicted response surface quadratic models for yield and purity of SDF from DRB was shown in Table 9.

The results indicated that X_1X_4, X_2X_4 and X_1^2 were not highly significant ($p>0.05$ n=3) for extraction yield, while X_1X_3, X_2X_4; X_1^2; X_2^2 and X_4^2 were not significant ($p>0.05$ n=3) for purity of SDF. Thus, the statistical analysis showed that the yield of SDF was significantly ($P < 0.05$) affected by the independent variable factors. Only the interactions between alcalase-NaOH concentration and alcalase-soaking time in NaOH solution showed no significant effect on the extraction the yield of SDF. However, the P-value of NaOH, amylase, alcalase and interaction between enzyme ratios were the smallest ($P < 0.0001$) which indicated that these factors highly affected the extraction of SDF.

The purity of SDF was affected by all single factor (all exhibited small P-value) and the P-value of NaOH concentration were the smallest (<0.0001). Therefore, NaOH concentration had more effect on the purity of SDF compared to other factors. Only the interactions of NaOH-amylase-DRB ratio, soaking time-alcalase-DRB ratio, quadratic NaOH concentration, soaking time and alcalase-DRB ratio did not significantly ($P > 0.05$ n=3) affected the purity of SDF. However; the purity was more affected by the interactions between the concentration and soaking time of NaOH solution and between enzyme concentrations (smallest P-value).

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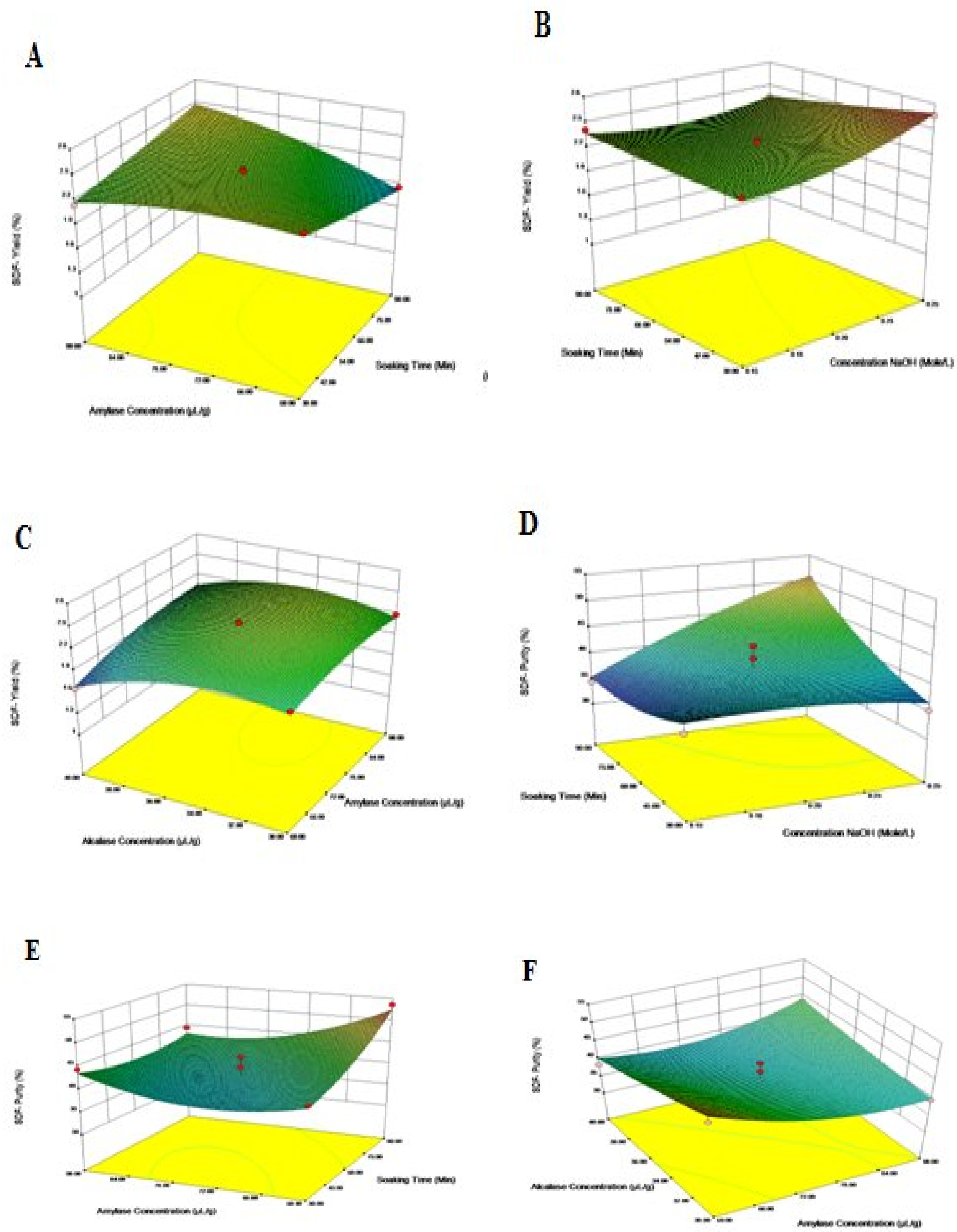

Figure 5. Response Surface Plots (3-D) showing effects of interaction of some variable factors on the yield and purity of SDF from defatted rice bran.

The response surface plots (3-D) of yield and purity of SDF from DRB affected by interaction of NaOH concentration and soaking time, the interaction of soaking time and α-amylase concentration and the interaction between ratios of α-amylase and alcalase to DRB are shown in the Figure 5.

The interaction between α-amylase-DRB ratio and soaking time showed that higher yield of SDF was reached when the ratio and time were increased while, longer soaking time combined with ratio in range improved the purity of SDF (Figure 5 A and E). However, the combination of a short soaking time with a low NaOH concentration gives a high yield of SDF at the same time the increasing of these parameters increased its purity (Figure 5 B and D). On the other hand the combination of the alcalase- DRB ratio in the range with increasing α-amylase- DRB ratio also leads to a high yield of SDF while, its purity increased with increasing of these parameters (Figure 5 C and F).

Based on this optimization, the optimum extraction conditions under which the yields and purities of IDF, SDF and TDF of DRB could be obtained with desirability of 73% were as follow: the concentration of NaOH 0.15 mol/L, the soaking time in the NaOH solution 64.03 min, the α-amylase – DRB ratio 0.68:100, and the alcalase – DRB ratio 3.52:100, respectively.

The optimum yields and purities of IDF SDF and TDF were:

IDF: yield: 27.44%, purity: 86.99%

SDF: yield: 2.35%, purity: 51.57%

TDF: yield 31.50%, purity: 79.71%

The prediction factor levels for optimal extraction of IDF, SDF and TDF were the following concentration of NaOH solution 0.15, soaking time in the NaOH solution 64.03 min, α-amylase-DRB ratio 0.68:100 and alcalase-DRB ratio 3.52:100.

The prediction yield and purity of IDF, SDF and TDF were: IDF-Yield 27.44%, IDF-Purity 86.99%, SDF-Yield 2.35%, SDF-Purity 51.57%, TDF-Yield 31.50% and TDF-Purity 79.71%.

The optimal levels of the factors of the extraction of IDF, SDF and SDF from DRB and the prediction responses (yield and purity) were confirmed with 95% confidence of these fiber fractions.

Conclusion

ANOVA showed most of the independent variable factors and their combinations significantly ($p<0.05$) affected the yield and purity of all three fiber fractions of DRB. The yield and purity of TDF and the purity of IDF were affected at the same level by all variable factors ($P < 0.0001$). However, the yield of SDF was more affected by NaOH concentration ($P < 0.0001$) while, its purity was less affected by soaking time ($P = 0.0012$) compared to other factors.

Highly correlated second-order polynomial model used to optimize the extraction yield of IDF, SDF and TDF from DRB showed that:

- The interactions between all variable factors except that between concentration of NaOH and amylase concentration, significantly affected the extraction yield of TDF,
- Only the interaction between concentrations of NaOH and alcalase has no significant effect on the yield of IDF,
- The yield of SDF was affected by the interactions between all single factors except that between NaOH–alcalase concentration and soaking time–alcalase concentration.

For the purity of IDF, SDF and TDF the polynomial model showed that:

- The purity of IDF and TDF has been affected by the interactions between all variable factors. While, the SDF purity was also affected by the interactions between these all factors except interactions between NaOH-amylase concentrations and soaking time-alcalase concentration.

The optimization study demonstrated that:

- The optimum extraction yield of TDF, IDF and SDF were 31.11, 26.88 and 2.69%, respectively
- The optimum purity of TDF, IDF and SDF were 81.84, 90, and 53.57%, respectively.

The optimum levels of independent extraction variables were the following: concentration of NaOH 0.15 mol/L, soaking time 64.03 min, α-amylase – DRB ratio 0.68:100, and the alcalase – DRB ratio 3.52:100.

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