OPTIMIZATION STUDY OF CITRUS WASTES SACCHARIFICATION BY DILUTE-ACID HYDROLYSIS

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The effects of time, acid concentration, temperature and solid concentration on dilute-acid hydrolysis of orange peels were investigated. A central composite rotatable experimental design (CCRD) was applied to study the individual effects of these hydrolysis factors and also their interdependence effects. The enzymatic hydrolysis of the peels by cellulase, β-glucosidase, and pectinase enzymes resulted in 72% dissolution of the peels, including 18.7% galacturonic acid and 53.3% of a total of glucose, fructose, galactose, and arabinose. Dilute-acid hydrolysis up to 210°C was not able to hydrolyze pectin to galacturonic acid. However, the sugar polymers were hydrolyzed at relatively low temperature. The optimum results were obtained at 116°C, 0.5% sulfuric acid concentration, 6% solid fraction, and 12.9 min retention time. Under these conditions, the total sugars obtained at 41.8% dry peels and 2.6% of total hexose sugars were further degraded to hydroxymethylfurfural (HMF). No furfural was detected through these experiments from decomposition of pentoses.

Keywords: Orange peel, Dilute-acid hydrolysis, Experimental design, Sugar optimization

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INTRODUCTION

Orange peel, a waste product from citrus processing factories, is partly used for cattle feed. However, the waste has about 66 million tons annual production (Pourbafrani et al. 2007) and a huge amount of it is still discarded to nature, causing several environmental problems (Tripodo et al. 2004). On the other hand, the peel contains various carbohydrate polymers, which make it an interesting choice for production of metabolites such as ethanol by appropriate microorganisms. An individual or combination of mechanical, chemical, and biological pretreatments, however, is required to break down cellulose, hemicellulose, and pectin polymers present in the cell walls of orange peels and convert them to their sugars' monomers (Grohmann et al. 1995; Grohmann and Baldwin 1992; Grohmann et al. 1994).

Enzymatic hydrolysis is an efficient method to release almost all carbohydrates present in the orange peels, but its application is hampered by the high cost of enzymes and the slow rate of the depolymerization reaction (Grohmann et al. 1995). Thus, development of a cost-effective method in which all or a high proportion of carbohydrates could be released will help to commercialize the processes using orange peels as raw materials. The advantages of dilute-acid hydrolysis for peel liquefaction and

releasing carbohydrates prior to enzymatic treatment have already been studied (Grohmann et al. 1995; Vaccarino et al. 1989a). There are different variables that might have impacts on the rate and extent of citrus peel hydrolysis by dilute-acid processes. Temperature, acid concentration (or pH), total solid fraction (TS), and time duration of the hydrolysis are the key variables in this process (Grohmann et al. 1995).

Statistical approaches in experimental design provide powerful tools to study and optimize several factors in a process simultaneously. Full factorial, partial factorial, and central composite rotatable designs (CCRD) are the most common techniques used for process analysis and modeling (Montgomery 2001). When the number of factors and responses increases, the last method (CCRD) is preferred, since it needs fewer tests than the other methods and gives almost as much information as other methods (Obeng et al. 2005). This method has already been applied for hydrolysis of a wide variety of materials to find the optimum conditions for corresponding processes (Canettieri et al. 2007; Rahman et al. 2007; Kunamneni and Singh 2005; Rodriguez-Nogales et al. 2007).

Factors that have previously been applied to investigate and optimize the hydrolysis of orange peels were limited to temperature and acid concentration, using one factor at a time rather than varying them simultaneously. This procedure does not allow studying the interaction between variables (if there is any), and thus the best condition that gives the highest yield of monomeric sugars and lowest yield of by-products cannot be achieved. Furthermore, decomposition of pentoses and hexoses produced to hydroxymethylfurfural (HMF) and furfural through the secondary hydrolysis reactions has not been studied.

The scope of the present work was to apply the central composite rotatable design to evaluate the variables that show significant effects in dilute-acid hydrolysis of orange peel. The main and interaction effects of variables on the yields of total liberated sugars and formation of HMF were studied. Based on the experimental design, a model was developed and the optimum conditions to attain the highest yield of carbohydrates and lowest yield of HMF were predicted and validated by an additional experiment. Furthermore, pectin resistance at high temperatures during acid hydrolysis was particularly investigated.

MATERIALS AND METHODS

Substrates and Enzymes

The orange peels used in this work were the residuals of Spanish orange obtained from Brämhults juice AB (Borås, Sweden) and stored frozen at -20 °C until use. The frozen peels were thawed and ground with a food homogenizer (ULTRA-TURAX,TP 18-20, Janke & Kunkel Ika-Labortechnik, Germany) to less than 2 mm in diameter. Total dry content of orange peel was determined by drying at 110 °C for 48 h. Three commercial enzymes, pectinase (Pectinex Ultra SP), cellulase (Celluclast 1.5 L) and β -glucosidase (Novozym 188), were provided by Novozymes A/S (Bagsvaerd, Denmark). Pectinase activity was measured by hydrolyzing 0.02% citrus pectin (P9135-Sigma) solution at 45 °C in 50 mM sodium acetate buffer at pH 4.8 (Wilkins et al. 2007). Cellulase activity was determined by hydrolyzing Whatman#1 filter paper in 50 mM

sodium acetate buffer at pH 4.8 and temperature of 45°C (Decker et al. 2003). The activities of pectinase and cellulase were measured as 283 international units (IU)/mg protein, and 0.12 filter paper units (FPU)/mg protein, respectively. The activity of β-glucosidase was reported as 2.6 IU/mg solid by the supplier.

Enzymatic and Acid Hydrolyses

Ground peels were added into 250 ml conical flasks containing 50 mM sodium acetate buffer at pH 4.8 to obtain 100 ml of peel/water slurry with solid fractions of 2, 4, 6, 8, and 10%. The slurries were then hydrolyzed by the enzymes at 45 °C and 140 rpm for 24 h in a shaker bath. For acid hydrolysis, ground peels were diluted with distilled water to obtain 100 ml of peel/water slurry with similar solid fractions as previously mentioned. Sulfuric acid (98%) was added to the slurries to reach final acid concentration of 0, 0.25, 0.5, 0.75, and 1% (v/v). Next, the slurries were heated in an autoclave at various temperatures of 100, 108, 116, 124, and 132 °C with different residence times of 5, 10, 15, 20, and 25 min according to the design of the experiment (Table 1). All samples were then cooled down to 85(±3) °C before removing them from the autoclave.

Acid Hydrolysis at High Temperature

A 10-L high-pressure reactor (Process & Industriteknik AB, Sweden) was used for acid hydrolysis. The reactor heated with direct injection of 60 bar pressure steam, which was provided from a power plant located in Borås, Sweden. The steam hydrolyzed the materials at desired temperature within the designed retention time. The materials were then explosively sent to an expansion tank to cool down for further processing and analyses. In these tests, two kilograms of orange peel/water slurry with solid concentration of 5-18% and acid concentration of 0-0.5% were loaded into the reactor and hydrolyzed for 10-30 min. at 140-210 °C.

Analytical Methods

An ion-exchange Aminex HPX-87P column (Bio-Rad, USA) was used at 85 °C for measuring glucose, galactose, arabinose, and fructose concentrations. Ultra-pure water was used as eluent at a flow rate of 0.6 ml/min. Concentrations of furfural, HMF and galacturonic acid were determined by an Aminex HPX-87H column (Bio-Rad, USA) at 60 °C using 5mM H_2SO_4 at a flow rate of 0.6 ml/min. A refractive index (RI) detector (Waters 2414, Milipore, Milford, USA) and UV absorbance detector at 210 nm (Waters 2487) were used in series. Furfural and HMF concentrations were analyzed from UV chromatograms, whereas the rest of the chemicals were quantified with a refractive index (RI) detector.

Statistical Analysis

The central composite rotatable experimental design method (CCRD) was chosen to determine the effect of four operating variables of the acid hydrolysis, including temperature (T), time, solid (TS), and acid concentration, and two response variables which were (a) yield of sugars defined as the sum of glucose, fructose, arabinose, and galactose produced per total peel dry mass, and (b) conversion of hexoses to HMF, hereafter called HMF yield. Selection of the factors and range of the variables were based

on the operating condition, which has a significant influence on the acid hydrolysis process according to previous works (Grohmann et al. 1995; Vaccarino et al. 1989b), as well as 40 preliminary experiments with 110-210 °C, 5-20%(w/w) solid concentration, 0-1%(v/v) acid concentration, and 5-30 min (data not shown).

Table 1: Coded (x_1, x_2, x_3 and x_4) and Respective Actual Levels (T, Solid%, Acid% and Time) in Experimental Design for Dilute-acid Hydrolysis of the Orange Peels by CCRD Method

Test no.	Coded level of variables				Actual level of variables			
	x_1	x_2	x_3	x_4	T(°C)	Solid(%)	Acid(%)	Time(min)
1	-1	-1	-1	-1	108	4	0.25	10
2	+1	-1	-1	-1	124	4	0.25	10
3	-1	+1	-1	-1	108	8	0.25	10
4	-1	-1	+1	-1	108	4	0.75	10
5	-1	-1	-1	+1	108	4	0.25	20
6	+1	+1	-1	-1	124	8	0.25	10
7	+1	-1	+1	-1	124	4	0.75	10
8	+1	-1	-1	+1	124	4	0.25	20
9	-1	+1	+1	-1	108	8	0.75	10
10	-1	+1	-1	+1	108	8	0.25	20
11	-1	-1	+1	+1	108	4	0.75	20
12	+1	+1	+1	-1	124	8	0.75	10
13	+1	+1	-1	+1	124	8	0.25	20
14	+1	-1	+1	+1	124	4	0.75	20
15	-1	+1	+1	+1	108	8	0.75	20
16	+1	+1	+1	+1	124	8	0.75	20
17	-2	0	0	0	100	6	0.5	15
18	+2	0	0	0	132	6	0.5	15
19	0	-2	0	0	116	2	0.5	15
20	0	+2	0	0	116	10	0.5	15
21	0	0	-2	0	116	6	0.0	15
22	0	0	+2	0	116	6	1.0	15
23	0	0	0	-2	116	6	0.5	5
24	0	0	0	+2	116	6	0.5	25
25	0	0	0	0	116	6	0.5	15
26	0	0	0	0	116	6	0.5	15
27	0	0	0	0	116	6	0.5	15
28	0	0	0	0	116	6	0.5	15
29	0	0	0	0	116	6	0.5	15
30	0	0	0	0	116	6	0.5	15

The number of tests required for CCRD is the sum of 2^k factorial runs with its origin at the center, 2k axial runs, and numbers of replicate tests at the center, where k is the number of the variables. In the current work, where the effects of four variables are to be evaluated, the recommended number of tests at the center point is six (Box and Hunter 1957), and therefore the total number of tests would be 30 as presented in Table 1. The values of the variables are coded to lie at ±1 for factorial points, 0 for the center points and ±2 for axial points (Obeng et al. 2005).

A software package called MINITAB® was used to evaluate and to fit the second-order model to these four independent variables according to the following equation:

$$Y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} b_{ii} x_i^{\,2} + \sum_{i}^{i<j} \sum_{j} b_{ij} x_i x_j + e \quad (1)$$

where Y is the dependent or response variable(s) to be modeled, x_i and x_j are the independent variables (factors), and b_i, b_{ii} and b_{ij} are the measures of the x_i , x_i^2 and x_ix_j effects, respectively. The variable x_ix_j represents the first-order interactions between x_i and x_j,and e is the error. When the response data are obtained from the test work, a regression analysis using the least-squares method is carried out to determine the coefficients of the response model, their standard errors, and significance. The effects were considered to be not statistically significant when the p-value was higher than 0.05 at the 95% confidence level (Obeng et al. 2005). The optimum values of the selected variables were obtained from the estimated variables in the model and by inspecting the response surface contour plots and MINITAB® optimizer.

RESULTS

Characterization of the Orange Peels

The dry matter content of the orange peels was measured at 20(±1.2)%. In order to find out the carbohydrate content of the peels, they were enzymatically hydrolyzed in shake flasks at 45 ˚C for 24 h. The respective loadings of pectinase, cellulase, and β-glucosidase were 1163 IU/g, 0.24 FPU/g, and 3.9 IU⁄g peel dry matter based on the optimized values previously reported (Wilkins et al. 2007). Yields of various sugars released in the enzymatic hydrolysis were not significantly influenced by increasing the concentration of peel solids. The average values of duplicate experiments are summarized in Table 2. The main carbohydrates after enzymatic hydrolysis were glucose, fructose, galactose, arabinose, and galacturonic acid (GA), accounting for 72% of the total solid content of the peels. These results are in agreement with those obtained from enzymatic hydrolysis of Argentina orange peel (Pourbafrani et al. 2007).

Table 2: Yields of the Sugars Released during Enzymatic Hydrolysis of the Orange Peels

Sugars	(%) of Total Solid
Glucose	27.1 ± 1.3
Fructose	14.1 ± 1.5
Galactose	5.0 ± 0.5
Arabinose	7.1 ± 0.6
Galacturonic acid	18.7 ± 1.5
Total	72

Dilute-acid Hydrolysis

Dilute-acid hydrolyses of the orange peels were performed according to the experimental design presented in Table 1. The results for yields of total sugars (Y_{TS}) and HMF (Y_{HMF}) are displayed in Table 3. The highest yield of sugars, 43.24%, was achieved in the 28th experiment at the center point of the experiments where 116 °C, 0.5% sulfuric

acid, 6% solid fraction and 15 min were applied. However, the more reliable value is the average of results of the 25th to 30th experiments, with a Y_{TS} value of 42.40±0.53 %. Furfural, a decomposition product of pentoses, was not detected in any of the experiments, while HMF was the main by-product. The highest yield of HMF was obtained at 9.03% when temperature, time, acid concentration, and solid fraction were 124°C, 20 min, 0.75% and 4%, respectively.

Table 3: Actual Values and Predicted Values for Sugar and Hydroxymethyl Furfural (HMF) Yield

Test no.	Y_{TS}[a] Actual	Y_{TS} Predicted	Y_{HMF}[b] Actual	Y_{HMF} Predicted
1	36.61	33.61	0.75	1.05
2	41.64	40.58	2.35	2.33
3	27.74	27.24	1.17	1.19
4	39.56	40.74	1.74	1.58
5	39.33	36.22	0.95	1.11
6	40.02	38.27	1.54	1.67
7	41.24	39.64	6.67	7.10
8	40.11	39.25	2.70	3.21
9	39.43	38.41	1.42	1.28
10	31.12	30.88	0.77	0.70
11	41.31	41.19	2.48	2.71
12	38.80	41.32	6.12	6.00
13	39.74	37.96	1.80	2.00
14	36.20	36.10	9.03	9.05
15	39.42	39.89	1.80	1.86
16	37.74	38.86	7.33	7.39
17	30.10	32.02	0.81	0.81
18	37.43	37.96	8.06	7.64
19	40.00	43.10	4.07	3.53
20	40.15	39.49	1.90	2.02
21	24.70	29.60	0.00	0.00
22	40.07	37.62	5.50	5.50
23	38.69	40.07	2.32	2.29
24	39.15	40.21	4.14	3.75
25	41.79	42.40	2.65	2.63
26	42.78	42.40	2.61	2.63
27	42.60	42.40	2.64	2.63
28	43.24	42.40	2.67	2.63
29	42.29	42.40	2.63	2.63
30	41.75	42.40	2.60	2.63

[a] Gram of total sugars per 100 gram initial dry matter

[b] As percentage of total hexoses converted to HMF

Dilute-acid Hydrolysis at High Temperature

Pectin was not hydrolyzed under the above hydrolysis conditions and no galacturonic acid was obtained in the hydrolyzates. Therefore, further experiments at higher temperature (140, 180, and 210 °C) for 10 and 30 min. and acid concentrations of zero, 0.05, and 0.5% and solid concentrations of 5, 6, 10, and 18% were carried out. However, no hydrolysis of pectin and no galacturonic acid in the hydrolyzates were

obtained. Furthermore, hydrolysis under these conditions did not improve Y_{TS} values than the results reported in Table 3.

Statistical Analysis of the Experimental Results

The results of statistical analysis including the estimated values of factors' coefficients, interactive terms, *t* from Student's *t*-test, and *p*-values are shown in Table 4. The larger magnitude of the *t*-value and the smaller magnitude of the *p*-value indicate more significance of the corresponding coefficient. Thus, temperature and acid concentration in linear and quadratic form were highly significant for the yield of total sugars ($p< 0.05$). Among the interactive terms, only interaction between temperature and acid concentration (X_{13}) was highly significant. For the second response variable (Y_{HMF}), temperature and acid concentration had the greatest effect on the formation of HMF, followed by total solid concentration and time. Temperature was the only significant quadratic term, but it showed interaction with all three other variables. Furthermore, there was an interaction between time and acid concentration regarding the yield of HMF (X_{34} in Table 4).

Table 4: Model Coefficients Estimated by Multiple Linear Regressions for Sugars and Hydroxymethyl Furfural (HMF) Yields

Factor	Yield of sugars			Yield of HMF		
	Coefficient	t-value	p-value	Coefficient	t-value	p-value
Intercept	42.41	42.70	0.000	2.63	19.95	0.000
X_1	1.48	2.99	0.009	1.70	25.86	0.000
X_2	-0.90	-1.82	0.089	-0.37	-5.72	0.000
X_3	2.00	4.04	0.001	1.48	22.45	0.000
X_4	0.03	0.07	0.944	0.36	5.51	0.000
X_1^2	-1.85	-3.99	0.001	0.39	6.45	0.000
X_2^2	-0.28	-0.59	0.560	0.03	0.58	0.569
X_3^2	-2.19	-4.73	0.000	-0.02	-0.37	0.717
X_4^2	-0.56	-1.21	0.242	0.09	1.57	0.136
X_{12}	1.01	1.66	0.116	-0.20	-2.47	0.026
X_{13}	-2.02	-3.33	0.005	1.06	13.11	0.000
X_{14}	-0.98	-1.61	0.126	0.20	2.52	0.024
X_{23}	1.00	1.66	0.118	-0.11	-1.37	0.189
X_{24}	0.26	0.42	0.677	-0.13	-1.70	0.110
X_{34}	-0.54	-0.88	0.388	0.26	3.31	0.005

* X_1, X_2, X_3 and X_4 are temperature, solid fraction, acid concentration and time, respectively. X_{ij} represents the first order interactions between X_i and X_j.

Regression analysis based on the coded variables on the experimental data was performed, and coefficients of the second-order models were calculated. Substitution of coefficients calculated and response variables in Eq. (1) resulted in the following empirical equations for yields of total sugars (Y_{TS}) and HMF (Y_{HMF}):

$$Y_{TS} = 42.41 + 1.48\,X_1 - 0.90\,X_2 + 2.00\,X_3 + 0.03\,X_4 - 1.85\,X_1^2 - 0.28\,X_2^2 - 2.19\,X_3^2 - 0.56\,X_4^2 + 1.01\,X_1X_2 - 2.02\,X_1X_3 - 0.98\,X_1X_4 + 1.00\,X_2X_3 + 0.26\,X_2X_4 - 0.54\,X_3X_4 \quad (2)$$

$$Y_{HMF} = 2.63 + 1.70\ X_1 - 0.37\ X_2 + 1.48\ X_3 + 0.36\ X_4 + 0.39\ X_1^2 + 0.03\ X_2^2 - 0.02\ X_3^2 + 0.09\ X_4^2 - 0.20\ X_1 X_2 + 1.06\ X_1 X_3 + 0.20\ X_1 X_4 - 0.11\ X_2 X_3 - 0.13\ X_2 X_4 + 0.26\ X_3 X_4 \quad (3)$$

The predicted values obtained from the model equations (Eqs. 2, 3) show good agreement with the actual values of both responses (Table 3). The regression for both responses was statistically significant at the 95% confidence level. The results of the second-order response surface model in the form of analysis of variance (ANOVA) for both responses are presented in Table 5. The suitability of the fitness can be checked by determination coefficients (R^2), which were 0.85 and 0.99 for the yields of total sugars and HMF, respectively. The R^2 statistic indicates the percentage of the variability of the optimization parameter that is explained by the model (Fannin et al. 1981) and, therefore, 15% and 1% of the total variations are not explained by the models developed for the corresponding yields of total sugars and HMF, respectively.

Table 5: Analysis of Variance (ANOVA) for Two Quadratic Models of the Total Sugar (TS) and HMF Yields

Responses	Source of Variation	Sum of squares	Degree of freedom	Mean square	F-value	Probability (p)
Y_{TS}	Regressions	489.67	14	34.97	5.91	0.001
	Residual	88.76	15	5.91		
	Total	578.43	29			
Y_{HMF}	Regressions	154.74	14	11.05	105.74	0.000
	Residual	1.56	15	0.10		
	Total	156.3	29			

Effects of Experimental Variables on Hydrolysis Results

The responses for yield of total sugars were depicted as three-dimensional surface plots of two factors, while the other factors were kept at center levels along with their corresponding contour plots in Figs. 1 and 2. The effects of temperature and acid concentration on the yield of sugars, when solid fraction and time were selected at their center points, are shown in Fig. 1a,b. At the lower levels of temperature, increase in acid concentration resulted in higher yield of sugars. Similarly, at low levels of acid concentration, increase in temperature had a positive effect on the sugar yield. However, at the higher levels of both temperature and acid concentration, the yield of sugars declined, certainly due to presence of the strong interaction between these two variables (Table 4). The maximum yield, 42.6%, was attainable only by conducting hydrolysis experiments in a limited region around 117 °C temperature and 0.6% acid concentration. Under these conditions, HMF yield was 3.4% (Fig. 1c). The effect of acid concentration at the lower levels of temperature on the formation of HMF was negligible, but the rate of sugar decomposition to HMF increased sharply with rising temperature as can be observed from closer counter lines at high temperature and acid concentration in Fig. 1c.

The effects of temperature and time on sugar yield are depicted in Fig. 1d,e. The maximum sugar yield, 42.6%, was obtained at 13 min and 120 °C, while the corresponding HMF yield was 3.4% (Fig. 1f). The minimum sugar yield of 27% was obtained when time and temperature were at the lowest levels, *i.e.* 5 min and 100 °C (Fig.

1e). There was an optimum level for time of hydrolysis where the highest yield of sugars could be obtained (Fig. 1,d,e).

The interdependence of temperature and total solid fraction is presented in Fig. 2a,b. In general, increase in total solid concentration led to a decreased yield of sugars. However, the extent of this reduction was lowered as the temperature was raised (Fig. 2a,b). Analysis of the effect of solid fraction and temperature on the second response variable (Y_{HMF}) showed that at the higher temperature, increase of the solid fraction resulted in a lower yield of HMF (Fig. 2c). The effect of variation of acid concentration and solid fraction is to a great extent similar to the effect of temperature and solid fraction, and therefore the same results can be inferred (data not shown). The highest yield of sugars, 43.1%, was achieved at 116 °C and 0.5% acid concentration with the lowest fraction of solid of 2% (Fig. 2b). The yield of HMF was around 3.5% under these conditions (Fig. 2c). Sugars' yield around 40% is attainable by precise adjustment of both temperature and acid concentration at 10% solid fraction (Fig. 2b).

Analysis of the effect of variation of acid concentration and time on sugar yield is presented in Fig. 2d,e. The surface plot for yield of carbohydrates reached a peak with increase in acid concentration and then declined with further increase in acid concentration. The optimum region that yielded maximum liberation of sugars (42.6%) corresponded to a time period of 15 min and acid concentration of 0.625%. The yield of HMF was around 3% in this region, and the rate of formation increased at higher acid concentration (Fig. 2f).

Based on the second-order models, numerical optimization was carried out to maximize the yield of carbohydrates and minimize yield of HMF, using the response optimizer in MINITAB®. The optimal values of test variables were calculated as 12.9 min, 116 °C, 0.5% v/v acid concentration, and 6% solid fraction, with the corresponding yields of 42.3% for carbohydrates and 2.5% for HMF. The validity of these results was confirmed through performing hydrolysis runs in triplicate under optimized conditions. The analyses show that the average yields of total sugars and HMF were 41.8% and 2.6%, respectively. These experimental findings were in close agreement with the model prediction. Sugars released by acid hydrolysis were equal to 79% of total sugars (excluding galacturonic acid) obtained by enzymatic hydrolysis (Table 2).

Analysis of concentration of various sugars released by carrying out hydrolysis under optimum conditions indicated that yields of glucose, fructose, galactose, and arabinose were 75%, 85%, 80%, and 80% of those from enzymatic hydrolysis, respectively. The major proportion of HMF formation resulted from decomposition of fructose, and there was a direct relationship between loss of fructose and formation of HMF when hydrolysis was performed at test variables higher than the optimum values. Thus, solid residue, which is mostly insoluble and crystalline cellulose, contains about 20% of the remaining, unhydrolyzed glucose and minor amounts of other sugars.

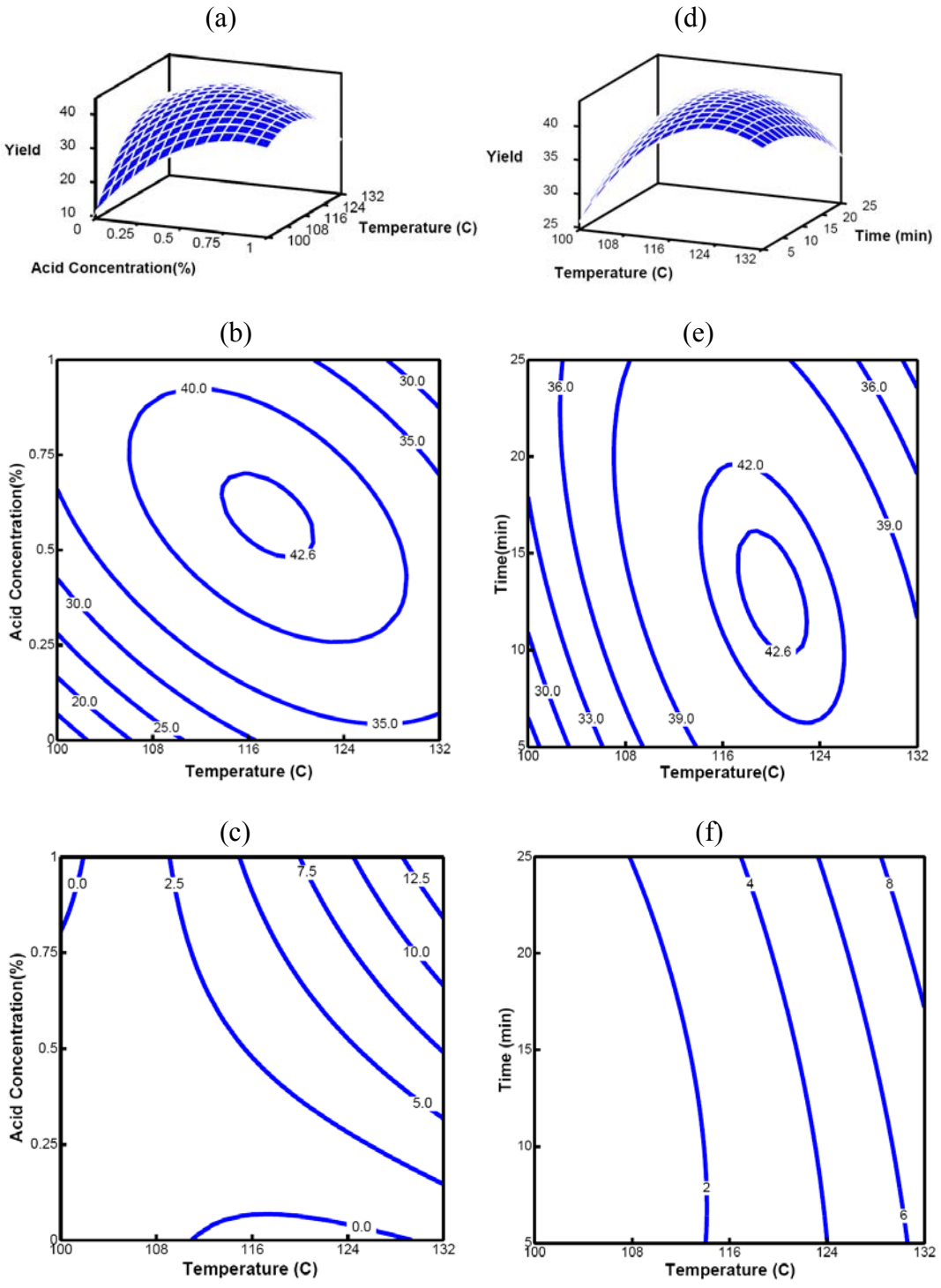

Fig. 1. Effect of temperature and acid concentration, time and temperature on the yield of total sugars (a,b,d,e) and HMF yield (c,f).

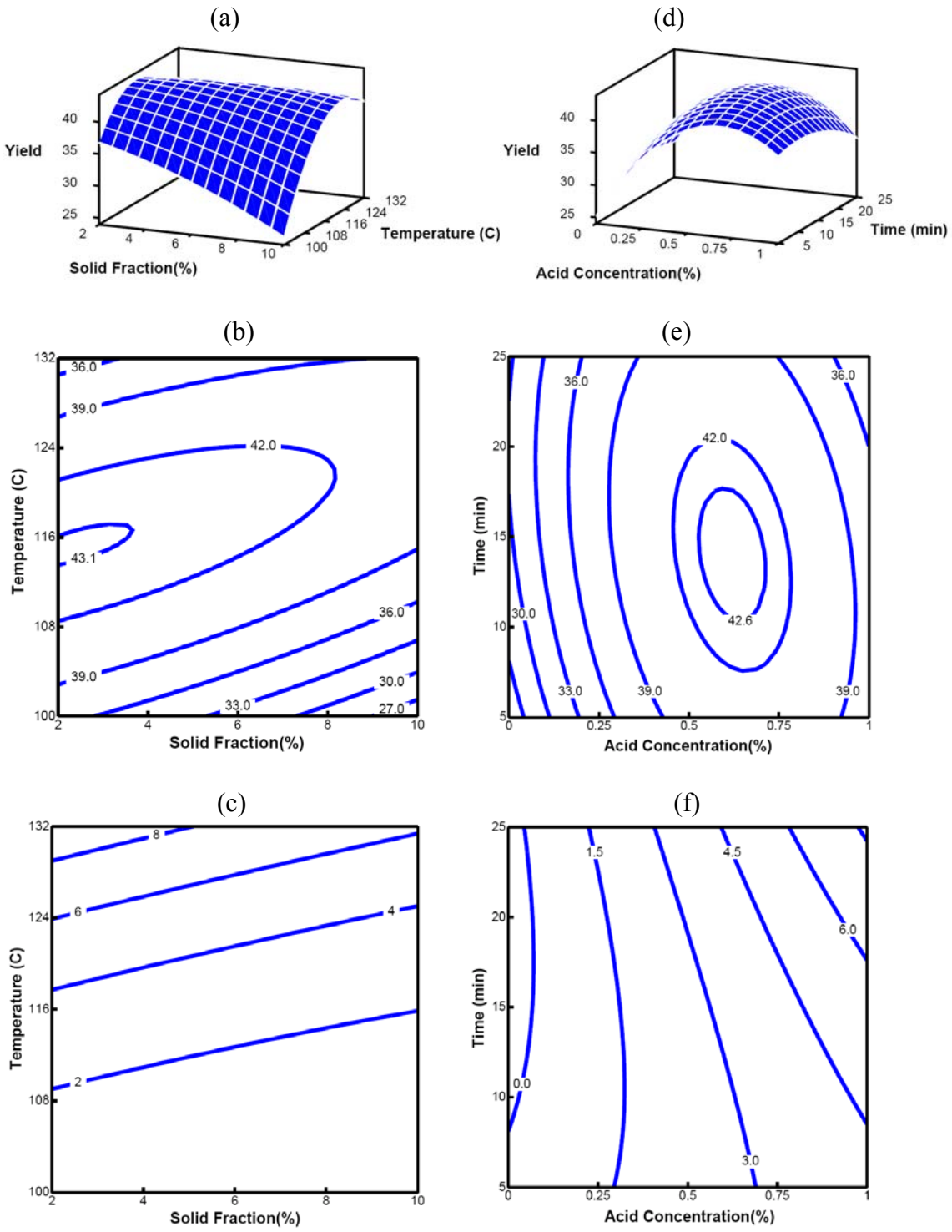

Fig. 2. Effect of temperature and solid faction, time and acid concentration on the sugar yield (a,b,d,e) and HMF yield (c,f).

DISCUSSION

Dilute-acid hydrolysis of citrus peel is an effective treatment prior to enzymatic hydrolysis that allows solubilization of a large fraction of the solid. The remaining residue contains cellulose and solubilized pectin mixed with soluble mono- and oligosaccharides from the peel. The pectin can be either recovered as a product or further hydrolyzed to galacturonic acid and minor amounts of other sugars. A wider range of temperature can be applied in acid than in enzymatic hydrolysis, and the time required for acid hydrolysis is much lower than that for enzymatic reaction (Grohmann et al. 1995). However, acid hydrolysis suffers from the formation of inhibitors such as furfural and HMF due to decomposition of liberated sugars through the secondary reactions (Bienkowski et al. 1987; Taherzadeh et al. 2000; Azhar et al. 1981). Fructose is a soluble sugar in orange peel, which starts to decompose at 120°C, and its rate of decomposition is even faster at higher temperature (Grohmann et al. 1995). These facts suggest that the conditions for dilute-acid hydrolysis and the variables affecting this process should be carefully selected and optimized to yield the highest rate and extent of depolymerization of the carbohydrate polymers and maximum release of sugars, while the formation of inhibitory compounds is minimized.

The results of the current work indicate that furfural was not formed in the range of test variables applied. Furfural is a decomposition product of pentoses, and its formation is a first-order reaction, where the reaction constant is affected by both acid concentration and temperature. Arabinose is the only pentose sugar present in the peel hydrolyzate that is released gradually during hydrolysis. Among the various pentose sugars exposed to the acid for furfural formation, arabinose showed the lowest reactivity, with a small reaction constant (Garrett and Dvorchik 1969). Therefore, lack of furfural formation is most probably due to stability of arabinose and its low concentration in hydrolyzate under the applied conditions.

Formation of HMF during dilute-acid hydrolysis is a sequential reaction where cellulose and hemicellulose are first hydrolyzed to their hexose monomers, followed by decomposition of liberated hexoses to HMF. The kinetics of these two reactions were studied for lignocellulosic materials, and the results indicated that these hydrolysis and decomposition reactions are both first-order reactions and possess rates of similar magnitude (Saeman 1945). These two reaction rates are influenced by temperature and acid concentration. The higher ratio of the first reaction rate constant compared to the second one increases the yield of total liberating sugars. The profile of total liberated carbohydrates vs. time passes through a maximum, and hence there is an optimal time at which sugar production reaches a maximum. This time is a function of constant values of both reactions. Elapsing time of hydrolysis longer than the optimal value enhances the speed of the second reaction, leading to a decrease in net total sugar liberation (Saeman 1945). This fact can be interpreted in another way from results of statistical analysis in Table 4. Time represents no significant effect on the yield of sugars (Y_{TS}), while its effect on the HMF yield is significant and shows interaction with both temperature and acid concentration. Thus, time is an important factor for the overall hydrolysis process to achieve the highest yield of total carbohydrates.

The effect of total solid concentration is significant only for the yield of HMF. The major fraction of HMF is formed from decomposition of the soluble sugars (mainly

fructose) that are available in the early period of hydrolysis.Concentration of these soluble sugars is proportional to the solid fraction, and this is a possible reason for the significance of the total solid fraction for the second response variable. The positive impact of total solid fraction on the reduction of HMF yield might be attributed to the presence of temperature and acid concentration gradients at the higher levels of solid fractions, which decreases the decomposition rate of sugars to the HMF in the bulk medium.

Pectin was not hydrolyzed in this work, and therefore no galacturonic acid was detected through the analyses. Despite the soluble nature of released pectin fragments, the glycosidic bonds between galacturonic acid units are probably too resistant to acid hydrolysis due to a combination of inductive and conformational effects. However, because of the low rate of pectin depolymerization, a much longer time of hydrolysis is probably required for partial release of galacturonic acid (Grohmann et al. 1995; Timell et al. 1965).

CONCLUSION

Hydrolysis of citrus processing waste was carried out with dilute acid, and the optimum conditions as well as interaction between influencing factors were investigated by utilizing a central composite rotatable design (CCRD). Among the linear terms, temperature and acid concentration were the most significant variables for both yields of sugars and HMF, and the latter was also influenced by the solid fraction and time. Time of hydrolysis longer than its optimized value proved to have a negative effect on the yield of sugars, mainly due to formation of HMF. At the higher levels of both temperature and acid concentration, total solid fraction had no significant effect on sugar yield. The maximum yield of sugars and minimum yield of HMF can be obtained under the optimum conditions as 41.8% and 2.6%, respectively.

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