Effects of α-Amylase and Glycerol Levels on the Composition Optimization of Poly(β-hydroxybutyrate-*co*-valerate)/Starch Blended Biodegradable Resin Analyzed with Response Surface Methodology

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ABSTRACT: Response surface methodology was used to analyze the effects of the enzyme level (X_2) and glycerol level (X_1) on objective attributes [water solubility index (WSI), water absorption index (WAI), and maximum loading (Y_3)] of a cornstarch/poly(β-hydroxybutyrate-*co*-valerate) blended composite. A rotatable central composite design was used to develop models for the objective responses. The experiments were run at barrel temperatures of 160, 160, 165, and 165°C, with a screw speed of 40 rpm and complete feeding (filling ratio = 1). Responses were most affected by changes in X_2 and to a lesser extent by X_1. Individual contour plots of the different responses were overlaid, and regions meeting the optimal WSI of 8.73%, WAI of 3.94 g of gel/g of dry weight, and Y_3 of 304.17 N were established at an X_2 of 5.43 g and an X_1 of 120.79 mL. These predicted values for the optimal process conditions were in good agreement with the experimental data. J Appl Polym Sci 120: 2571–2578, 2011

Key words: additives; biodegradable; extrusion

INTRODUCTION

Huge amounts of generated waste have made acquiring sufficient areas for landfill sites more difficult. The lack of capacity of waste landfills has become a serious problem, especially in urban areas, and the effective reduction or decomposition of waste is now necessary to secure adequate landfill capacity. Among the several compounds in waste landfill sites, plastics are estimated to fabricate approximately 20–30% of the volume of municipal solid waste landfill sites.[1] Because plastics are resistant to microbial degradation, they remain in landfill sites semipermanently. Plastic waste is recognized as one of the most troublesome categories of waste, and the disposal of plastic waste has been blamed for shortening the life of landfill sites. In response to this problem of plastic waste in landfills, biodegradable plastics (BPs), which have been designed to be easily degraded by microorganisms and to be absorbed by the natural environment or by waste landfills, are gaining public endorsement as a possible alternative to petroleum-derived plastics.[2]

Poly(hydroxyl alkanoate)s have received considerable attention in recent years as biodegradable alternatives to synthetic thermoplastics. Copolymers of hydroxybutyrate and hydroxyvalerate, commonly referred to as poly(β-hydroxybutyrate-*co*-valerate)s (PHBVs), have been produced and marketed by Zeneca Biopolymers. Because of the high cost of PHBV relative to thermoplastics, substantial attention in recent years has been paid to the incorporation of starch and other low-cost components into PHBV-based composites.[3–8] Starch is an attractive filler for PHBV because of its low cost and inherent biodegradability.

The use of starch in PHBV composites is hampered by the low degree of adhesion between the starch granules and the polymer matrix. The resulting loss in tensile strength and elongation compared to the unfilled polymer limits the maximum amount of starch that can be incorporated.[4,7] Shogren[5] showed that precoating the starch with poly(ethylene oxide) improves the tensile strength and elongation, presumably because of improved adhesion from poly(ethylene oxide)–PHBV interactions. Kotnis et al.[6] showed that the degree of adhesion between starch granules and PHBV was poor but could be mitigated via appropriate formulation and processing techniques to obtain materials with commercially useful properties.

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TABLE I
Levels and Levels of the RSM Design with Different Operating Conditions

	Coded		Uncoded	
Treatment	X_2	X_1	X_2 (mL)	X_1 (mL)
1	1	1	2.17	98.79
2	−1	1	7.83	98.79
3	−1	−1	2.17	141.21
4	1	−1	7.83	141.21
5	0	1.414	1	120
6	0	−1.414	9	120
7	1.414	0	5	90
8	−1.414	0	5	150
9	0	0	5	120
10	0	0	5	120
11	0	0	5	120
12	0	0	5	120
13	0	0	5	120

Another approach to improving adhesion between starch fillers and PHBV is by the grafting of a functional monomer onto the starch. Starch graft copolymers have been extensively studied and are easily prepared by free-radical polymerization with unsaturated monomers.[9]

The effectiveness of response surface methodology (RSM) in the development and optimization of cereal products has been highlighted by different authors.[10–14] The basic principle of RSM is to relate the product properties of regression equations that describe interrelations between the input parameters and product properties. Examples for appropriate applications of this technique in food extrusion include the optimization of complex products and the properties of process variables.[15]

In this study, our objective was to optimize the formulation of the enzyme level (X_2) and glycerol level (X_1) for a cornstarch/PHBV blended resin by extrusion technology. The well-established functional property of amylase acted as a chain attacker of the α-1,4 link of starch, whereas the glycerol acted as a good destructuring–plasticizing agent.[16,17] The objective of this study was to optimize the formulation of raw compositions, that is, X_2 and X_1, for the production of a cornstarch/PHBV blended composite by RSM.

EXPERIMENTAL

Materials

Corn starch (a fine white powder with a 10 wt % moisture content and containing 30 wt % amylose and 70 wt % amylopectin) was obtained from Hong Chi Co., Ltd. (Taiwan, Republic of China). PHBV [Y1000, D300P [5% beta-hydroxyvalerate (HV)], Chinese Tianan Biology Material Co., Ltd.] was purchased from the Ming Guan Instrument Co., Ltd. (Taiwan, Republic of China). The concentration of amylase (from human saliva, Sigma reference A0521, Sigma Chemical Co., St. Louis, MO) was similar to the one usually found in human blood plasma (50 U/L). The enzyme solution had an activity of 0.35 mg/U/min at pH 6.9 and 20°C/g of soluble starch. To stabilize the amylase, 1 mL of calcium chloride, obtained from human saliva (Sigma reference A0521), was used. X_1 (analytical reagent grade) were obtained from Sigma.

Extruder

A single-screw extruder made by Yea Jing Machinery Co., Ltd. (Taiwan, Republic of China), with a screw compression ratio of 2.8, a dihedral angle (ψ) of 2.8, and a length-to-diameter ratio of 32 was used. An electrical resistive heater (220 V, 1700 W) heated the temperature zone at the die. A single-screw volumetric feeder fed the formulas (Table I). A $19.1 \times 2.93\ \text{mm}^2$ rectangular single-hole die was used to give continuous extrudates. The experiments were run at barrel temperatures of 160, 160, 165, and 165°C with a feed rate of 25 g/min and a screw speed 35 rpm.

Experimental design

This study was based on the hypothesis that the water solubility index (WSI or Y_1), water absorption index (WAI or Y_2), and maximum loading (Y_3) were functionally related to the specific composition, and we attempted to fit multiple-regression equations describing quality composition responses.[18,19] Table I lists the levels of the RSM design with different amylase and X_1s.

A central composite design (Table II) was adopted.[18,19] In this design, for two variables, the size of the experiment was reduced with the 2^k factorial (2^2); this made the total number of experiments equal to 13 instead of 50 with full factorial design.[18,19] Experiments

TABLE II
Box–Behnken Arrangement and Responses

	Response		
Treatment	WSI (%)	WAI (g of gel/g of dry weight)	Y_3 (N)
1	4.77 ± 0.47	4.08 ± 0.11	271.00 ± 5.14
2	6.89 ± 1.03	4.07 ± 0.23	265.47 ± 3.57
3	8.20 ± 0.31	4.04 ± 0.15	284.16 ± 10.26
4	9.81 ± 0.68	3.84 ± 0.31	299.68 ± 7.24
5	5.22 ± 0.44	4.07 ± 0.35	292.88 ± 6.95
6	10.31 ± 0.23	3.87 ± 0.07	319.17 ± 2.14
7	6.82 ± 0.22	4.16 ± 0.34	245.00 ± 12.35
8	8.33 ± 0.13	3.96 ± 0.14	240.02 ± 2.61
9	8.45 ± 0.56	3.96 ± 0.24	308.09 ± 9.42
10	8.18 ± 1.21		305.09 ± 1.16
11	8.59 ± 0.78	3.94 ± 0.21	307.43 ± 7.64
12	8.42 ± 0.84	3.95 ± 0.34	292.52 ± 12.94
13	8.62 ± 0.97	3.96 ± 0.11	299.34 ± 6.57

TABLE III
Regression Model of the Variance of the Process Variables to the Extrudate WSI

Term	Coefficient	SE coefficient	T	p
Constant	8.4528	0.1374	61.503	0.001
X_1	0.7323	0.1087	6.740	0.001
X_2	1.6940	0.1087	15.591	0.001
$X_1 \times X_1$	−0.5038	0.1165	−4.323	0.003
$X_2 \times X_2$	−0.4069	0.1165	−3.492	0.010
$X_1 \times X_{2s}$	−0.1275	0.1537	−0.830	0.434

$S = 0.3073$; $R^2 = 97.8\%$; adjusted $R^2 = 96.3\%$; $T = t$-value; SE = standard error.

were randomized to minimize the effects of unexplained variability in the observed responses due to external factors. The function was assumed to be approximated by a second-degree polynomial equation:

$$Y_k = b_{k0} + \sum_{i=1}^{2} b_{ki}X_i + \sum_{i=1}^{2} b_{ii}X_i^2 + \sum_{i \neq j=1}^{2} b_{kij}X_iX_j \qquad (1)$$

where Y_k is the response variable; X_i and X_j represent the coded independent variables; b_{k0} is the value of the fitted response at the center point of the design, that is, point (0, 0), and b_{ki}, b_{ii}, and b_{kij} are the linear, quadratic, and cross-product regression terms, respectively.

The increments of variation for each variable spaced around the center point levels and the responses are presented in Table II. Feed compositions were coded for solutions of the multiple-regression (prediction) equations.[18,19]

The design depended on the symmetrical selection of variation increments with respect to the center composition. These levels of variation were chosen to be within the range of reasonable formulations, and the increments were carefully selected because interpretation of the results was valid only within the experimental limits.[18,19] The levels selected were also based on the conclusions of several previous studies[20–24] that were important for cornstarch/PHBV blended composites. As a matter of fact, the optimal processing variables for each response did not fall exactly in the same region in the two-dimensional space formed by the composition levels. Moreover, those constraints were set such that all responses (WSI, WAI, and Y_3) met their optimal acceptable region with the same composition levels. Therefore, we assumed that WSI should be more than 3.93 but less than 3.95, that WAI should be more than 8.7 but less than 9.0, and that Y_3 should be more than 304 N but less than 306 N.

WAI and WSI were measured with a technique developed for cereals.[25] The ground extrudate was suspended in water at room temperature for 30 min, gently stirred during this period, and then centrifuged at 3000 × g (g is the relative centrifugal force) for 15 min. The supernatant was decanted into an evaporating dish of known weight. WSI was taken as the weight of dry solids in the supernatant and expressed as a percentage of the original weight of sample. WAI was taken as the weight of gel obtained after removal of the supernatant per unit weight of the original dry solids. Determinations were made in triplicate.

Y_3 testing was carried out on the tension test specimens. We used 5 mm/min for the BPs, and the tests were carried out in conformity with ISO 294, ISO 527 (TS 1396), and TS 720.[26–28] The maximum tensile stress is the stress that a material can sustain without fracture. One calculates it by dividing the maximum load applied during the tensile test by the original cross sectional area of the sample. These tests were carried out at least five times for each specimen, and the results were averaged arithmetically.

Analysis of data

The regression analysis was conducted with the stepwise variable selection backward elimination procedure[18,19] to fit the model represented by eq. (1) to the experimental data. Optimization of the polynomial thus fitted was performed by numerical techniques with the mathematical optimizer procedure of Minitab 14.2 software package (Minitab. Inc., USA), which deals with constraints. The mapping of the fitted response surfaces was achieved with the internal microprogram of Minitab 14.2. The response surfaces and contour plots for these models were

TABLE IV
Analysis of Variance of the Process Variables to the Extrudate WSI

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	p
Regression	5	29.9010	29.9010	5.9802	63.32	0.001
Linear	2	27.2468	27.2468	13.6234	144.24	0.001
Square	2	2.5892	2.5892	1.2946	13.71	0.004
Interaction	1	0.0650	0.0650	0.0650	0.69	0.434
Residual error	7	0.6611	0.6611	0.0944		
Lack of fit	3	0.5408	0.5408	0.1803	5.99	0.058
Pure error	4	0.1204	0.1204	0.0301		
Total	12	30.5621				

Seq = sequential; DF = degree of freedom; SS = sum of squares; MS = mean squares.

TABLE V
Regression Model of Variance of the Process Variables to the Extrudate WAI

Term	Coefficient	SE coefficient	T	p
Constant	3.95587	0.01752	765.902	0.001
X_1	−0.06732	0.004083	−16.486	0.001
X_2	−0.06129	0.004083	−15.011	0.001
$X_1 \times X_1$	0.05024	0.04379	11.474	0.001
$X_2 \times X_2$	0.00548	0.004379	1.252	0.251
$X_1 \times X_2$	−0.05202	0.005775	−9.008	0.001

$S = 0.01155$; $R^2 = 99.0\%$; adjusted $R^2 = 98.3\%$; T = t-value; SE = standard error.

plotted as a function of two variables. The overlapping of the contour plots was done to take into account the three responses for their optimal values corresponding to two variables at a time.

RESULTS AND DISCUSSION

Diagnostic checking of the fitted model

The regression analyses for different models indicated that the fitted quadratic models accounted for more than 90% of the variations in the experimental data; this was highly significant. Multiple-regression equations were generated that related WSI, WAI, and Y_3 to coded levels of the variables.[18] The developed models are indicated as follows, whereas the terms of the equations were based on the p value (p < 0.01 or 0.05) evaluation, shown in Tables III–VIII:

$$\text{WSI}\ (Y_1) = 8.4528 + 0.7323X_1 + 1.694X_2 - 0.5038X_1^2 - 0.4069X_2^2 - 0.1275X_1X_2\ (R^2 = 0.963)$$

$$\text{WAI}\ (Y_2) = 3.95587 - 0.06732X_1 - 0.06129X_2 + 0.05024X_1^2 + 0.00548X_2^2 - 0.05202X_1X_2\ (R^2 = 0.983)$$

$$Y_3 = 302.503 + 0.368X_1 + 10.57X_2 - 28.545X_1^2 + 3.214X_2^2 + 5.263X_1X_2\ (R^2 = 0.932)$$

All the main effects, including linear and quadratic, and interaction of effects were calculated for each model. The regression coefficients are shown in Tables III, V, and VII, and the analyses of variance obtained for all of the models are shown in Tables IV, VI, and VIII. The correlation coefficients for WSI, WAI, and Y_3 (R^2's = 0.963, 0. 983, and 0. 932, respectively) were very high for a response surface.

First, Table III indicates that X_2 had positive linear but negative quadratic effects on WSI. X_1 had positive linear but negative quadratic effects on WSI as well. Table IV shows that the multiple-regression analysis of the WSI model was significant; however, the interaction of the variables of the model was not significant.

Second, Table V shows that X_2 had negative linear but positive quadratic effects on WAI. X_1 had negative linear but positive quadratic effects on WAI as well. Table VI shows that the multiple-regression analysis of the WAI model was significant as was the interaction between the variables of the model.

Finally, Table VII illustrates that X_2 had positive linear and quadratic effects on Y_3. X_1 had positive linear but negative quadratic effects on Y_3. The interaction of X_2 and X_1 had a high positive effect on Y_3. Table VIII shows that the multiple-regression analysis of the Y_3 model was significant; however, the interaction between the variables of the model was not significant.

Analysis of variance

Once a model was selected, an analysis of variance was calculated to assess how well the model represented the data. The analyses of variance for different responses are presented in Tables IV, VI, and VII. To evaluate the goodness of the model, coefficient of variation (S) and F value tests were performed. As a general regulation, S should be not greater than 10%.[18,19]

In this study, the coefficients of variation for WSI, WAI, and Y_3, were 0.3073, 0.01155, and 6.426%, respectively. Also, the F values for both responses were significant at the 95% level, as shown in Tables IV, VI, and VIII. The contour and response surface

TABLE VI
Analysis of Variance of the Process Variables to the Extrudate WAI

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	p
Regression	5	0.094699	0.094699	0.018940	141.99	0.001
Linear	2	0.066307	0.066307	0.033154	248.56	0.001
Square	2	0.017569	0.017569	0.008784	65.86	0.001
Interaction	1	0.010823	0.010823	0.010823	81.14	0.001
Residual error	7	0.000934	0.000934	0.000133		
Lack of fit	3	0.000733	0.000733	0.000244	4.86	0.080
Pure error	4	0.000201	0.000201	0.000050		
Total	12	0.095633				

Seq = sequential; DF = degree of freedom; SS = sum of squares; MS = mean squares.

TABLE VII
Regression Model of Variance of the Process Variables to the Extrudate Y_3

Term	Coefficient	SE coefficient	T	p
Constant	302.503	2.874	105.269	0.001
X_1	0.368	2.272	0.162	0.876
X_2	10.570	2.272	4.653	0.002
$X_1 \times X_1$	−28.545	2.436	−11.717	0.001
$X_2 \times X_2$	3.214	2.436	1.319	0.229
$X_1 \times X_2$	5.263	3.213	1.638	0.145

$S = 6.426$; $R^2 = 96.0\%$; adjusted $R^2 = 93.2\%$; $T = t$-value; SE = standard error.

plots for both responses are shown in Figures 1–3. From analyses of the residuals (data not shown), we concluded that they were randomly distributed around zero, and there was no evidence of outliers.[18,19]

Conditions for optimal responses

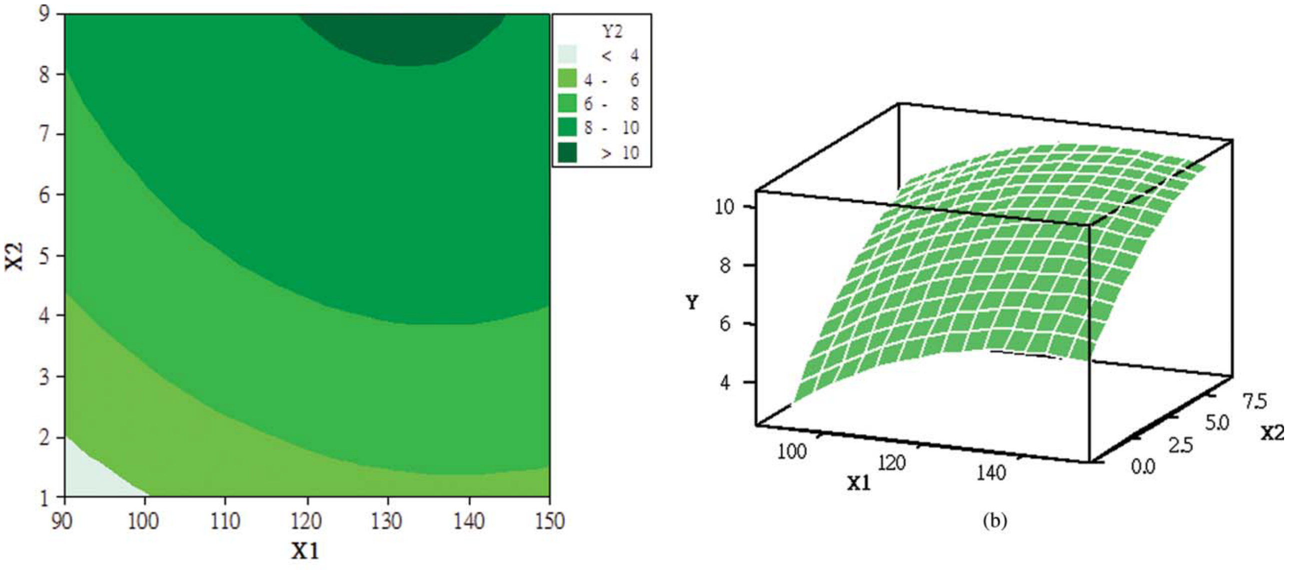
The direction in which to change the variables to optimize WAI, WSI, and Y_3 was usefully indicated by the models. The multiple-regression equation for Y_1 was solved for the optimal WSI (8.73%), Y_2 was solved for the optimal WAI (3.94 g of gel/g of dry weight), and Y_3 was solved for the optimal Y_3 (304.17 N). The optimal conditions needed to achieve the previous responses are presented in Figure 5 (shown later). The optimal values of WSI, WAI, and Y_3 for all of the variables were close to the middle of the experimental range; this indicated the validity of the selection of the variables range, and the models were accepted because of their significance at $p < 0.01$. The response surface models were obtained by the selection of three variables, and the one remaining had the value that led to the optimal response in the equations of Y_1, Y_2, and Y_3. Some selected surfaces are presented in Figures 1–3.

Along the horizontal axis in Figure 1, it is shown that an increase in X_2 had a positive parabolic effect on WSI. Meanwhile, X_1 had a positive parabolic effect on WSI as well. The optimum value of WSI was very close to the middle of the experimental region. An increase in the water solubility in starch-

TABLE VIII
Analysis of Variance of the Process Variables to the Extrudate Y_3

Source	DF	Seq SS	Adjusted SS	Adjusted MS	F	p
Regression	5	7014.6	7014.6	1402.91	33.98	0.001
Linear	2	894.9	894.9	447.43	10.84	0.007
Square	2	6008.9	6008.9	3004.46	72.77	0.001
Interaction	1	110.8	110.8	110.78	2.68	0.145
Residual error	7	289.0	289.0	41.29		
Lack of fit	3	116.7	116.7	38.90	0.90	0.514
Pure error	4	172.3	172.3	43.08		
Total	12	7303.6				

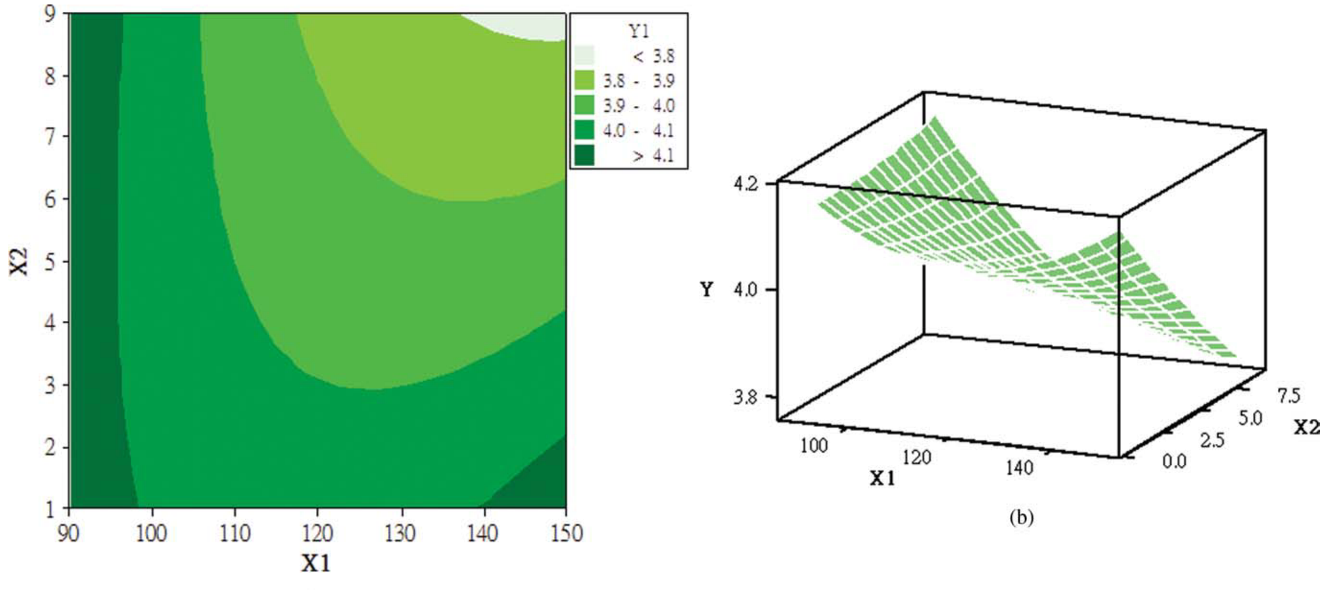
Seq = sequential; DF = degree of freedom; SS = sum of squares; MS = mean squares.



(a)

(b)

Figure 1 Contour and response surface plots of extrudate WSI. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



(a)

(b)

Figure 2 Contour and response surface plots of extrudate WAI. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

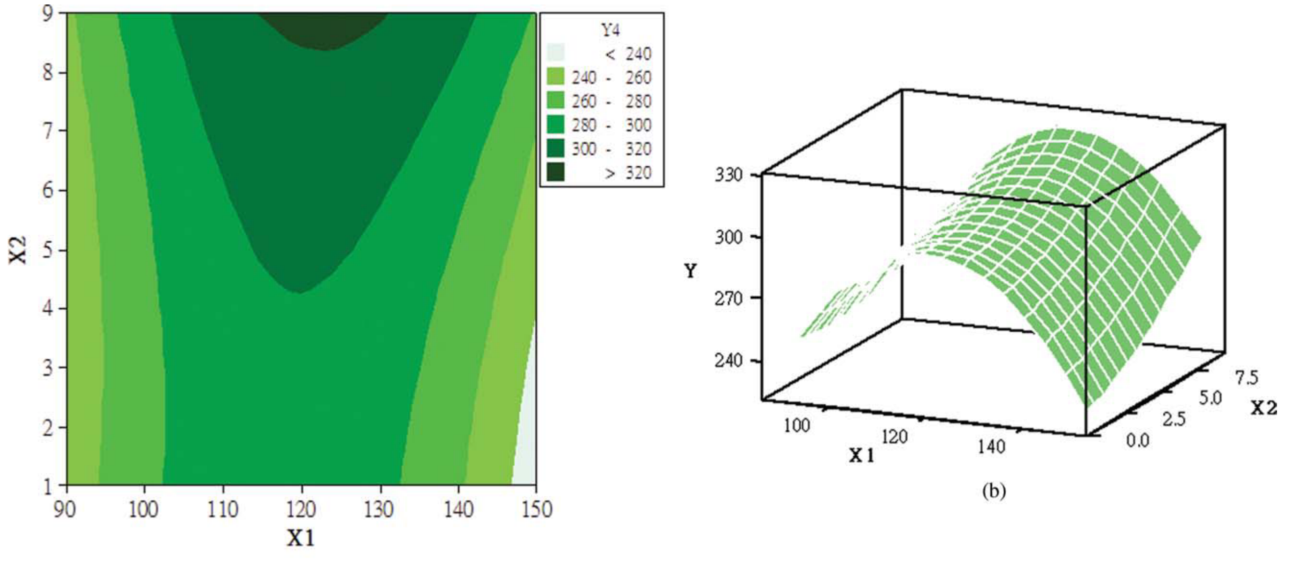
based extruded resin seemed to be due to starch gelation, which occurred at 20% water content, high temperature, and high shearing force during the extrusion.

Figure 2 shows that an increase in X_2 had a negative linear effect on WAI. However, X_1 had a negative parabolic effect on WAI. The optimum value of WAI was very near to the bottom of experimental region. Gomez and Aguilera[29] reported that an increase in the starch level increased the amount of —OH functional groups; this resulted in an increase in water absorption.

As shown in Figure 3, X_2 had a positive linear effect on Y_3, and the optimum was near the middle value of the level. However, X_1 also had a parabolic effect on Y_3. Glycerol increased the moisture content of the extrudates and resulted in an increase in Y_3.

Superimposition of the contour plots of responses

The areas of optimal performance were located by the superimposition of the contour graphs for WSI, WAI, and Y_3 for composition levels that established the limits of acceptable quality for each factor.



(a)

(b)

Figure 3 Contour and response surface plots of extrudate Y_3. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Because the optimal processing variables for each response did not fall exactly in the same region in the two-dimensional space formed by the compositions levels, constraints were set such that all responses (WSI, WAI, and Y_3) met their optimal acceptable region with the same composition levels. We assumed that WSI should be more than 3.93 but less than 3.95, that WAI should be more than 8.7 but less than 9.0, and that Y_3 should be more than 304 but less than 306 N.[24,30–32] Superimposing the individual contour plots for the response variables resulted in the identification of a region (shown by the white area); this satisfied all constraints and runs at barrel temperatures of 160, 160, 165, and 165°C, with a feed rate of 25 g/min and screw speed of 35 rpm, as shown in Figure 4. However, it may not be advisable to set the experimental conditions very rigidly, and therefore, a moderation level was given to each process variable and response, as shown in Figure 5. Hence, the final optimal conditions were established for WSI of 8.73%, WAI of 3.94 g of gel/g of dry weight, and Y_3 of 304.17 N at an X_2 of 5.43 g and an X_1 of 120.79 mL, respectively, as shown in Figure 5. The optimal conditions were experimentally tested, and we obtained a WSI of 8.70%, a WAI of 4.06 g of gel/g of dry weight, and a Y_3 of 302.77 N.

CONCLUSIONS

The results from this study confirm that the system of WSI, WAI, and Y_3 of the cornstarch/PHBV blended composite could be effectively optimized with RSM and with a minimum number of experiments. Also, computerized computations, model building, and the generation of three-dimensional graphs and contours were effective in simplifying the complexity of the preparation of the cornstarch/PHBV blended composites with restricted levels of enzyme and glycerol used. According to the optimal conditions given for the variables, the process could be scaled up for industrial production, and the cornstarch/PHBV blended composite would be suitable for BP applications.

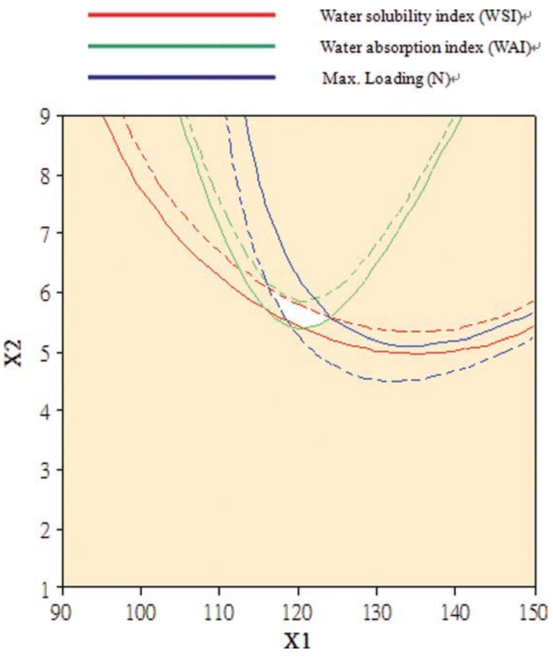


Figure 4 Optimal operating conditions from the contour plots. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

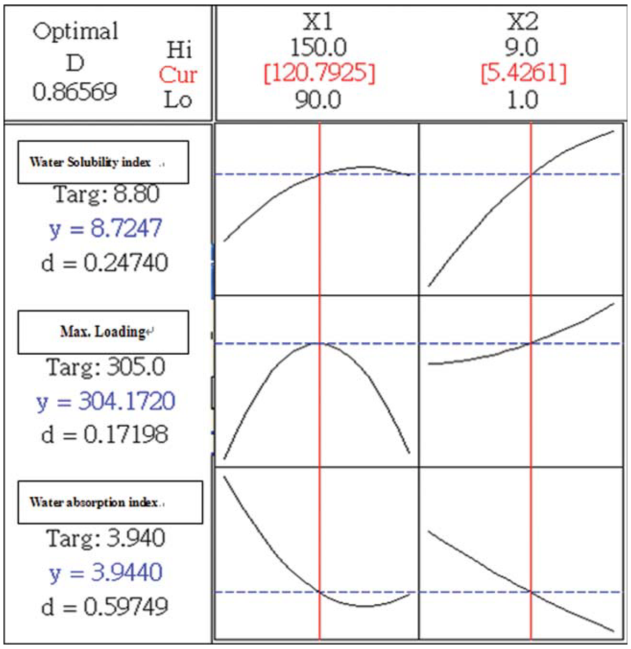


Figure 5 Predicted responses values by the optimal formula. y = predicted value; d = desirability. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

References

1. Franklin Associates, Ltd. Characterization of Municipal Solid Waste in the United States; final report prepared for U.S. Environmental Protection Agency; EPA Office of Solid Waste: Washington, D.C., 1990.
2. Ishigaki, T.; Sugano, W.; Nakanishi, A.; Tateda, M.; Ike, M.; Fujita, M. Chemosphere 2004, 54, 225.
3. Yasin, M.; Holland, S. J.; Jolly, A. M.; Tighe, B. J. Biomaterials 1989, 10, 400.
4. Ramsay, B. A.; Langlade, V.; Carreau, P. J.; Ramsay, J. A. Appl Environ Microbiol 1993, 59, 1242.
5. Shogren, R. L. J Environ Polym Degrad 1995, 3, 75.
6. Kotnis, M. A.; O'Brien, G. S.; Willett, J. L. J Environ Polym Degrad 1995, 3, 97.
7. Koenig, M. F.; Huang, S. J. Polymer 1995, 36, 1877.
8. Owen, A. J.; Koller, I. Polymer 1996, 37, 527.
9. Kardos, J. L. In Molecular Characterization of Composite Interfaces; Ishida, H.; Kumar, G., Eds.; Plenum: New York, 1985; p 1.
10. Malcolmson, L. J.; Matsuo, R. R.; Balshaw, R. Cereal Chem 1993, 70, 1.

11. Saxena, D. C.; Rao, P. H. Int J Food Sci Technol 1996, 31, 345.
12. Shelke, K.; Dick, J. W.; Holm, Y. F.; Loo, K. S. Cereal Chem 1990, 67, 572.
13. Sushma, T.; Saxena, D. C. Lebensm-Wiss Technol 2000, 33, 354.
14. Vaisey-Genser, M.; Ylimaki, G.; Johnson, B. Cereal Chem 1987, 64, 50.
15. Vainionpaa, J.; Malkki, Y. In 7th World Congress of Food Science and Technology; Ghee, A. H., Ed.; Singapore Institute of Food Science and Technology: Singapore, 1987; p 338.
16. Colonna, P.; Doublier, J. L.; Melcion, J. P.; Monredon, F. D.; Mercier, C. Cereal Chem 1984, 61, 538.
17. Linko, P.; Mattson, C.; Linko, Y. Y.; Antila, J. J Cereal Sci 1984, 2, 43.
18. Chatterjee, S.; Price, B. In Regression Analysis by Example; Bradley, R. A.; Kendall, D. G.; Watson, G. S., Eds.; Wiley: New York, 1977; p 201.
19. Cocharan, W. G.; Cox, G. M. In Experimental Designs; Bradley, R. A.; Kendall, D. G.; Hunter, J. S.; Watson, G. S., Eds.; Wiley: New York, 1957; p 335.
20. Thakore, I. M.; Desai, S.; Sarawade, B. D.; Devi, S. Eur Polym J 2001, 37, 151.
21. Kiatkamjornwong, S.; Thakeow, P.; Sonsuk, M. Polym Degrad Stab 2001, 73, 363.
22. Demirgoz, D.; Elvirs, C.; Mano, J. F.; Cunha, A. M.; Piskin, E.; Reis, R. L. Polym Degrad Stab 2000, 70, 161.
23. Kim, M. Carbohydr Polym 2003, 54, 173.
24. Santayanon, R.; Wootthikanokkhan, J. Carbohydr Polym 2003, 51, 17.
25. Bhatnagar, S.; Hanna, M. A. Trans ASAE 1995, 38, 567.
26. Plastic-Injection Molding of Test Specimen of Thermoplastics Materials; ISO 294; ISO: Geneva, Switzerland; 1995.
27. Plastic Determination of Tensile Properties; ISO 527 (TS 1396); ISO: Geneva, Switzerland, 1966.
28. Plastic Determination of Izod Impact Strength of Rigid Materials; ISO 180 (TS 1005); ISO: Geneva, Switzerland; 1982.
29. Gomez, M. H.; Aguilera, J. M. J Food Sci 1983, 48, 378.
30. Coffin, D. R.; Fishman, M. L.; Ly, T. V. J Appl Polym Sci 1996, 61, 71.
31. Lawton, J. W.; Fanta, G. F. Carbohydr Polym 1994, 23, 275.
32. Avella, M.; Errico, M. E.; Lauriennao, P.; Martuscelli, E.; Raimo, M.; Rimedio, R. Polymer 2000, 41, 3875.