Application of Response Surface Methodology to the Study of Methyl Glucoside Polyester Synthesis Parameters in a Solvent-Free System

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Response surface methodology (RSM) and 3-level-3-factor fractional factorial design were used to evaluate the effects of synthesis parameters, including reaction time (4 to 8 h), temperature (110 to 130 °C), and substrate molar ratio of fatty acid methyl esters (FAME) from soybean oil to methyl glucoside (4:1 to 6:1) on the percent molar conversion to methyl glucoside polyester (MGPE), utilizing 15 g of methyl glucoside as the reactant in a solvent-free system. All synthesis variables (reaction time, temperature, and substrate molar ratio) exhibited significant effects on percent molar conversion to MPGE in the experimental range. Optimization of the synthesis reaction was suggested by ridge max analysis to compute the estimated ridge of optimum response for increasing radii from the center of the original design. Based on the ridge max analysis, optimum conditions were: reaction time 6.3 h, synthesis temperature 123.8 °C, and substrate molar ratio 5.9:1. The predicted molar conversion was 55.68% (i.e., 15 g methyl glucoside yielded 56.5 g MGPE) at the optimum point.

Keywords: *Contour plot; methyl glucoside polyester; optimization; response surface*

INTRODUCTION

The food industry has introduced the use of fat substitutes in foods as reduced or zero calorie ingredients. Many fat replacers are based on starches, gums, and emulsifiers that thicken with water to give a feeling of thickness in the mouth, and some are based on proteins broken into micrometer-size particles on the tongue to feel like fat (Kirschner, 1997). Those fat replacers are limited by heat stability, texture, taste, and marketing. Olestra (sucrose polyester, SPE) exhibits functional and physical properties that resemble conventional triglycerides but contributes no calories to the diet (Bernhardt, 1988; Kester, 1993). Olestra is approved for use replacement of up to 100% of the fat used in the preparation of savory snacks (Federal Register, 1996). However, Olestra might trigger gastrointestinal symptoms such as loose stools, abdominal gas, cramping, and flatulence (Schlagheck, 1997). In addition, the apparent viscosities of Olestra are significantly greater than that of salad oil (Akoh and Swanson, 1990; Shieh et al., 1996b). Methyl glucoside polyester (MGPE), a potential fat substitute consisting of a methyl glucoside molecule with four fatty acids attached to the hydroxyl groups, may serve as a potential alternative fat substitute (Akoh and Swanson, 1991). An optimized process for high-yield synthesis of MGPE will benefit food manufacturers and processors.

Preliminary feeding trials with mice suggest that MGPE as a potential fat substitute in the diet is potentially safe and may be useful for weight reduction (Akoh and Swanson, 1991). The physical properties and functions are similar to conventional triglycerides (Akoh and Swanson, 1989). If MGPE is used in food as a partial or complete replacement for oil and fats, then it is imperative that its oxidative stability be maximized and deterioration be minimized. Tenox TBHQ has been shown be the best antioxidant for improving the oxidative stability of MGPE (Akoh, 1994).

Albano-Garcia et al. (1980) reported a solvent-less synthesis of methyl glucoside esters of coconut fatty acids in the presence of the catalysts 5% anhydrous potassium soap and 0.5% sodium metal at temperatures of 145−148 °C. The yield ranged from 36−78%. Akoh and Swanson (1989) synthesized methyl glucoside polyesters by a solvent-free system of methyl glucoside with a fatty acid methyl ester (FAME) of long-chain fatty acids. At a molar ratio of methyl glucoside tetraactate to FAME of 1:5, acceptable yields as high as 99.5% were achieved with 2% Na as the catalyst and heating at 98 to 105 °C. Boutte and Swanson (1994) modified the soap method using a rotary evaporator in a laboratory scale experiment and suggested approximate reaction parameters. The synthesis of MGPE was implemented at 120 to 125 °C for 6 to 8 h with a FAME to methyl glucoside ratio of 6:1. However, no optimum conditions were specified nor were any combined effects of reaction parameters described.

Our objectives were to better understand the relationships between the synthesis factors such as reaction time, synthesis temperature, and reactants' molar ratio and the response (synthesis yield); to determine the optimum synthesis conditions for MGPE production, using RSM and ridge max analysis.

MATERIALS AND METHODS

Experimental Design. A 3-level-3-factor experimental design with 3 replicates at the center point was used in this

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Table 1. Fractional Factorial Design, Observed Experimental Data, and Predicted Values for 3-Level-3-Factor Response Surface Analysis

	factors					
treatment no.[a]	reaction time (h) x_1	reaction temperature (°C) x_2	substrate molar ratio (FAME:MG) x_3	MGPE weight (g)	observed molar conversion[b] (%)	predicted molar conversion[d] (%)
1	8(1)$_c$	130(1)	5(0)	47.61	46.91	46.42
2	8(1)	110(−1)	5(0)	14.49	14.00	13.34
3	4(−1)	130(1)	5(0)	19.44	19.15	20.09
4	4(−1)	110(−1)	5(0)	12.40	12.71	12.69
5	8(1)	120(0)	6(1)	49.05	48.33	49.35
6	8(1)	120(0)	4(−1)	27.47	27.07	27.45
7	4(−1)	120(0)	6(1)	42.55	41.92	41.53
8	4(−1)	120(0)	4(−1)	9.46	9.33	8.29
9	6(0)	130(1)	6(1)	63.27	62.33	61.79
10	6(0)	130(1)	4(−1)	32.51	32.03	32.12
11	6(0)	110(−1)	6(1)	40.13	39.54	39.44
12	6(0)	110(−1)	4(−1)	13.64	13.44	13.98
13	6(0)	120(0)	5(0)	35.33	34.80	33.51
14	6(0)	120(0)	5(0)	31.17	30.71	33.51
15	6(0)	120(0)	5(0)	35.56	35.03	33.51

[a] The treatments were run in a random order. [b] The theoretical % molar conversion is calculated from the weight of the final product divided by 101.50 (full conversion), assuming an MGPE containing four fatty acids. [c] The values (−1), (0), and (1) are coded levels. [d] The predicted values are derived from eq 2.

study, requiring 15 treatments (Box and Behnken, 1960; Shieh et al., 1996a). The reaction factors and levels were chosen to investigate the optimal synthesis conditions on the basis of the work of Boutte and Swanson (1994). The three independent variables (x_i), levels, and experimental design in terms of coded and uncoded are shown in Table 1.

Materials. Soybean oil was purchased locally (Changhua, Taiwan). α-Methyl glucoside was purchased from Aldrich Chemical Co. (Milwaukee, WI) and naphthoresorcinol (1,3-naphthalenediol) from Eastman Kodak Co. (Rochester, NY). Supelco Redi-coats G silica gel (20 × 20 × 0.25 cm) TLC plates were purchased from Supelco, Inc. (Bellefonte, PA). All organic solvents, sodium hydroxide, hydrochloric acid, potassium hydroxide, and potassium carbonate were purchased from Merck Chemical Co. (Darmstadt, Germany).

Synthesis and Purification. MGPE was synthesized using FAME derived from soybean oil by the procedure used by Akoh and Swanson (1988). The average molecular weight of the soybean oil FAME was 294.3, based on fatty acid composition as determined by gas liquid chromatography (GLC). Basically, the synthesis procedure of MGPE was according to the patented procedure of McCoy et al. (1989), as modified by Boutte and Swanson (1994). In this study, no solvent was used during the synthesis procedure. The reaction apparatus was described by Shieh et al. (1996a), except that a 2-L three-necked round-bottom flask with a magnetic stirring bar was attached to an evaporator as the reactor. Heat was supplied by an external, electrically heated and thermostatically controlled heat mantle to maintain a constant temperature. In a typical synthesis, milled potassium hydroxide (2.5 wt % of FAME and methyl glucoside) and FAME were added to the 2-L three-necked round-bottom flask first and stirred at room temperature for 30 min, after which 15 g methyl glucoside was added. Reactants were heated to 85 °C for 15 min at atmospheric pressure to form soap. Then, the mixture was heated to selected temperatures (110, 120, or 130 °C) to promote the transesterification reaction at full vacuum (∼100 mTorr). After heating to the experimental temperature, 0.5% (w/w) potassium carbonate was added to catalyze the MGPE reaction. At the end of the experimental time (4, 6, or 8 h), the reaction was cooled to 80 °C and neutralized with 2−3 mL of concentrated acetic acid. The crude product was washed three times with 95% ethanol at 60 °C, and five times with 1.5 L water (70 °C). Then, the MGPE was dissolved in hexane, stirred and bleached with decolorizing carbon (∼20%, w/w) twice at 100 °C for 1.5 h under a medium vacuum (∼100 mmHg) in a rotary evaporator. Charcoal particles were removed by filtering the MGPE solution through a 0.5 μm filter. A KDL-4 short-path distillation apparatus (UIC, Joliet, IL) was used to remove excess FAME and methyl glucoside esters with a low degree of substitution. Conditions for short-path distillation were as follows: evaporator heat of 150 °C, condenser temperature of −2.5 °C, wiper speed of 500 rpm, vacuum less than 0.01 mmHg. The MGPE was passed through the short-path distillation twice, but it was not steam deodorized (Shieh et al., 1996a).

Table 2. Analysis of Variance for Synthesis Variables Pertaining to the Response Percent Molar Conversion

source	df	sum of square	prob > F
model	9	3308.534445	0.0000
linear	3	2703.019753	0.0007
quadratic	3	403.888979	0.0034
cross product	3	201.625713	0.0000
lack of fit	3	5.234463	0.8304
pure error	2	11.851974	
total error	5	17.086437	
R^2 [a]	0.995		

[a] R^2 is the coefficient of determination.

MGPE Confirmation and Analysis. Thin layer chromatographic (TLC) analysis of the MGPE was conducted according to the method of Akoh and Swanson (1987). The solvent system used as a mobile phase for MGPE development was: petroleum ether/diethyl ether/acetic acid, 75:25:1 (v/v/v). The MGPE was detected by spraying the plate with naphthoresorcinol reagent (specific for nonreducing sugars and sugar fatty acid esters) and visualized as purple-violet spots on a white background when heated at 105 °C for 10 min. The fatty acid composition of the MGPE was obtained by transesterification with 1.0 M methanolic NaOH. Esterified fatty acids of soybean oil and fatty acid composition of the MGPE were analyzed by GLC.

Statistical Analysis. The data were analyzed by means of the Statistical Analysis System (SAS) (SAS Institute, Inc., 1990). The experimental data (Table 1) were analyzed by the response surface regression (RSREG) procedure with a lackfit option to fit the following second-order polynomial equation:

$$Y = \beta_{k0} + \sum_{i=1}^{3}\beta_{ki}x_i + \sum_{i=1}^{3}\beta_{kii}x_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{kij}x_i x_i \qquad (1)$$

where Y is the response (% molar conversion); β_{k0}, β_{ki}, β_{kii}, and β_{kij} are constant coefficients, and x_i the uncoded independent variable. Canonical analysis was one part of the RSREG SAS output. The RIDGE MAX option was used to compute the estimated ridge of optimum response for increasing radii from the center of the original design. Individual contour plots were created by holding one of three variables constant.

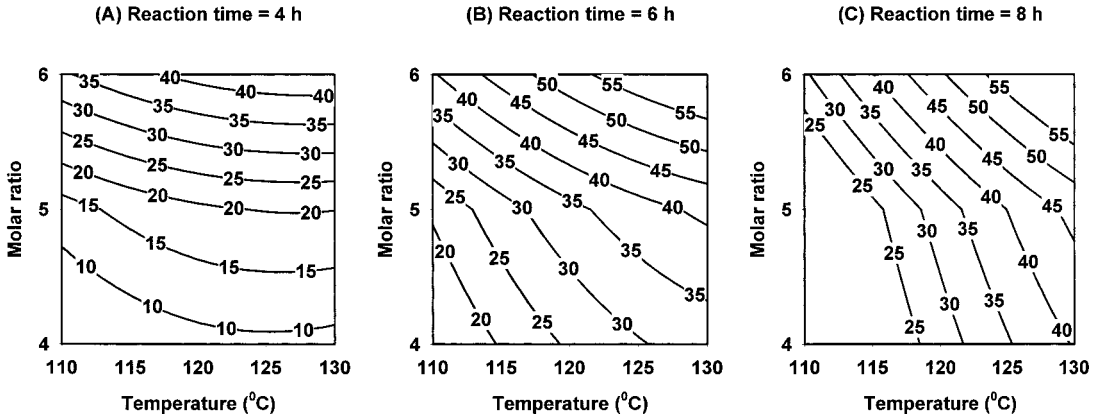

Figure 1. Contour plots showing response behavior of synthesis temperature and substrate molar ratio under constant reaction time. The numbers inside the contours represent theoretical percent molar conversion to MGPE at given reaction conditions.

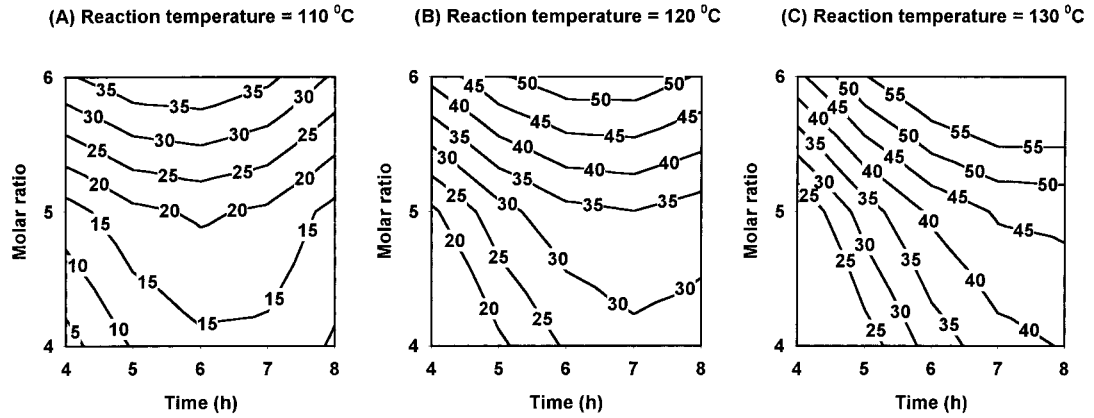

Figure 2. Contour plots showing response behavior of reaction time and substrate molar ratio at constant synthesis temperatures.

RESULTS AND DISCUSSION

Response surface methodology (RSM) is a useful statistical technique for investigating complex synthesis processes and is used to optimize the synthesis of Olestra (Shieh et al., 1996a). In this study, the percent molar conversion of MGPE was defined as the short-path distilled MGPE product weight divided by 101.5, under the assumption that the theoretical molecular weight of MGPE with four long-chain fatty acids was 1314.0, as determined from fatty acid composition by GLC analysis. With a full percent molar conversion, 15 g methyl glucoside (0.077 mol) synthesizes 0.077 mol of MGPE, which is 101.5 g.

The RSREG procedure for SAS was employed to fit the second-order polynomial eq 1 to the experimental data—percent molar conversions (Table 1). Among the various treatments, the greatest molar conversion (62.33%) was treatment 9 (6 h, 130 °C, and molar ratio 6:1), and the smallest conversion (only 9.33%) was treatment 8 (4 h, 120 °C, and molar ratio 4:1). From the output of RSREG, the second-order polynomial eq 1 is given below:

$$Y = -221.817 - 4.747x_1 + 4.801x_2 - 49.520x_3 - 1.945{x_1}^2 - 0.026{x_2}^2 + 5.919{x_3}^2 + 0.321x_1x_2 - 1.417x_1x_3 + 0.105x_2x_3 \quad (2)$$

The analysis of variance (Table 2) indicated that the second-order polynomial model was adequate to represent the actual relationship between the response percent molar conversion and the significant variables, with no significant lack of fit ($p = 0.830$) and a satisfactory coefficient of determination ($R^2 = 0.995$). Furthermore, the overall effect of the three synthesis variables on the percent molar conversion of MGPE was further analyzed by a joint test, which tested the hypothesis that parameters involving one particular factor are zero. The results revealed that the reaction time (x_1), synthesis temperature (x_2), and substrate molar ratio (x_3) were important factors with statistically significant overall effects ($p < 0.001$) on the molar conversion of MPGE. Because the real relationship between response and factors was either unknown or too complex to be useful, the simple empirical second-order equation was assumed to be adequate.

The relationships between reaction factors and response can be better understood by examining the series of contour plots generated by holding constant either the reaction time (Figure 1), synthesis temperature (Figure 2), or substrate molar ratio (Figure 3). The reaction time significantly affected the percent molar conversion (40 to 50%) from 4 to 6 h, but between 6 and 8 h the increase in percent molar conversion was minimal (~50%). The percent molar conversion (Figure 2) increased rapidly at 110 to 120 °C, but did not increase significantly between 120 and 130 °C reaction temperatures. The substrate molar ratio (Figure 3) exhibited the most significant effect on the percent molar conversion because the percentage yield at molar ratio 6:1 (Figure 3C) was significantly higher than the others (Figure 3A,B).

Optimum synthesis conditions were suggested by canonical analysis and ridge max analysis as described by SAS (SAS Institute, Inc., 1990) and Shieh et al. (1996a). By canonical analysis, not only was the station-

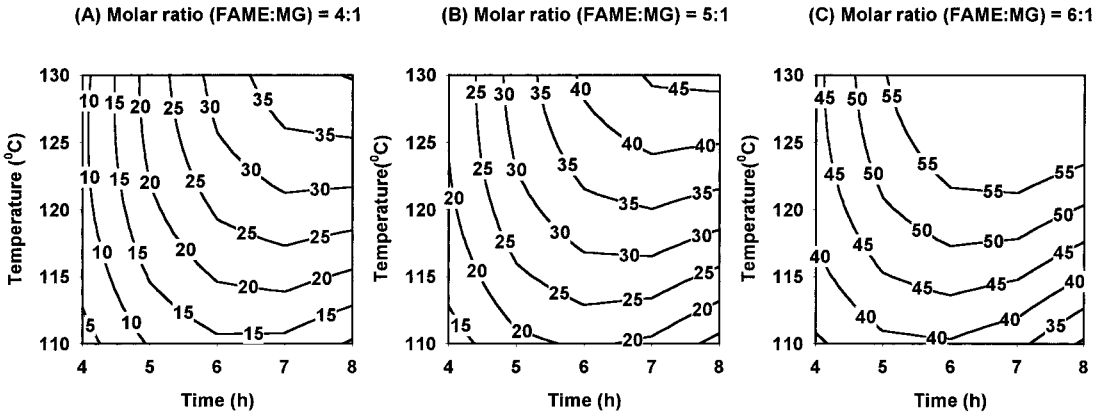

Figure 3. Contour plots showing response behavior of reaction time and synthesis temperature under constant substrate molar ratios.

Table 3. Estimated Ridge of Maximum Response for Variable % Molar Conversion

coded radius	estimated response (% conversion)	standard error	x_1 (h)	x_2 (°C)	x_3 (FAME:MG)
0	33.517	1.067	6.000	120.00	5.000
0.2	37.305	1.054	6.123	121.037	5.160
0.4	41.363	1.021	6.206	121.923	5.335
0.6	45.751	0.991	6.260	122.658	5.522
0.8	50.513	1.002	6.293	123.263	5.716
1	55.679	1.110	6.310	123.764	5.913

ary point (reaction time 6.3 h, synthesis temperature 171 °C, and molar ratio 4:1) located outside the experimental region but also the molar conversion was just 61% at extremely high synthesis temperatures. The canonical analysis based on the stationary point resulted in the following equation:

$$Y = 61.859 + 6.069\ W_1^2 - 1.067\ W_2^2 - 9.462_3^2 \quad (3)$$

where W_1, W_2, and W_3 are eigenvalues based on coded data and Y is the molar conversion of MGPE (%). The mixed signs of the eigenvalues indicated that the predicted response surface of the stationary point is shaped like a saddle. Because the canonical analysis resulted in a saddle point, the estimated surface did not exist with a unique optimum. Therefore, this analysis could not be used to identify the optimum conditions. The ridge analysis method computes the estimated ridge of optimum response for increasing radii from the center of the original design. The ridge analysis (Table 3) indicated that the maximum molar conversion (55.6 ± 1.1%) was at 6.3 h, 123.7 °C, and the 5.9:1 substrate molar ratio was found at the distance of the coded radius 1.0. The stationary point (61.86%) derived from canonical analysis was higher than the maximum point (55.68%) from ridge max analysis, but an extremely high reaction temperature was needed to reach the high yield. Furthermore, a higher synthesis temperature may increase the darkness of the MPGE and, consequently, make decolorization difficult. Our objective was to obtain economically optimal synthesis conditions, not necessarily maximum yield from an industrial viewpoint. Therefore, the ridge max point is recommended as the optimal synthesis condition in this study. Our work is primarily confirmatory of the results obtained by Boutte and Swanson (1994).

Observed and predicted values from the prediction model for the yielded percent molar conversion are also presented in Table 1. Chi-square tests indicated that the observed values were significantly the same (p-value > 0.90, degree of freedom = 14) as the predicted values for the model (Ott, 1988). This indicates that the parameter estimates presented in eq 2 can be used to predict the percent molar conversion of MGPE.

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