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Full Length Research Paper

Statistical modeling and optimization of enzymatic milk fat splitting by soybean lecithin using response surface methodology

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Fat splitting using enzyme is important in reducing the fat content of human body. Hence in the present work, fat splitting of milk butter was attempted using soybean lecithin. A 3-level central composite design was used for optimization of process variables such as of initial butter, initial enzyme concentration, temperature and digestion time on percentage fat splitting using soybean lecithin. The influence of the variables on percentage fat splitting was represented by a second order polynomial model using results obtained in 31 experimental runs of central composite design. The various effects of the factors were studied by student's t-test and Fisher's F-test for analysis of variance. The model fitted well with high coefficient of determination (R^2 = 0.904) justified an excellent correlation between variables and percentage fat splitting. The predicted optimum conditions for the maximum percentage fat splitting was initial milk butter, 6% (w/v), initial enzyme concentration, 4% (w/v), temperature, 40°C and digestion time 89.99 min for 7.38% (w/w) of experimental percentage of fat splitting.

Key words: Lecithin, milk butter, fat splitting, optimization, central composite design.

INTRODUCTION

Fat splitting is generally defined as the process of obtaining fatty acids from triglycerides by catalyzing action of an acid, alkaline or enzyme as catalyst in presence of water at relatively low temperature. Thus, fat splitting may be classified as acid splitting, basic splitting, continuous high temperature splitting and enzymatic splitting (Muckerheide, 1952). Enzymatic fat splitting is advantageous because it operates at mild conditions and more specific in reaction. The rate of enzymatic fat splitting depends upon the various factors. Establishing the optimum conditions and understanding the activation energy are important to analyze the characteristics of the enzymatic reactions.

The tissues of an adult human contain relatively constant amounts of protein and carbohydrate. Although there are more than twenty fatty acids occurring in the foods, the common animal body fats composed chiefly of the glycerides of palmitic, stearic and oleic acids and in addition generally small amounts of myristic and linolic acids. Both unsaturated fat and saturated fat are found in a variety of foods; studies have found that these fats are not created equally. Unsaturated fats can be beneficial to the heart, whereas saturated fats lead to obesity and could be harmful to heart. Unsaturated fat is a liquid at room temperature, differs from other fats in that it contains one or more double-bonds between carbon atoms in its structure (Barnebey and Brown, 1948; Cown, 1955; David, 1995). Obesity has been linked to raise incidence of premature death as well as several serious medical conditions, including type-2 diabetes, insulin resistance, heart disease, high blood cholesterol, high blood pressure, and stroke. Obesity is also a risk factor in higher rates of certain types of cancer, as well as fatty liver disease, vascular disorders, thrombosis, obstructive sleep apnea, musculoskeletal problems and gastro-esophageal reflux. Abdominal obesity is associated with insulin resistance syndrome and cardiovascular disease (Schwartz et al., 1962; Wallnoefer et al., 1973; Golay and Bobbioni, 1997; Bruha and Marecek, 2000; Romieu and

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Lajous, 2009).

Lecithins are biologically active additives to food and forages created from the valuable components of soya oil. Lecithin is a phosphatidylcholine. Lecithin is present in soybeans, seeds of sunflower, and germs of wheat. Soybean lecithin is a complex mixture of phospholipids, glycolipids, triglycerides, sterols and small quantities of fatty acids, carbohydrates and sphingolipids. Soya lecithin has been popular through the years as a supplement taken for the purpose of fighting cholesterol, supporting liver health, and promoting weight loss (Brook et al., 1986). Phosphatidylcholine facilitates the emulsification of fat into the tiniest particles within the nanosphere, enabling the absorption and transportation of fat. After subcutaneous injections of phosphatidylcholine into fat tissue, the adipocytes burst and phosphatidylcholine increases the secretion of triaglycerolrich lipoproteins, which leads to the dissolution of fat by producing an emulsion of nano sized monoglycerides that is transported into the liver and metabolized by beta-oxidation, in the citric acid cycle. Phosphatidylcholine is also known to protect the liver through the regeneration of liver cells in cases of fat liver hepatitis and alcoholic hepatic steatosis. The choline and inositol in lecithin protect against hardening of the arteries and heart disease by promoting normal processing of fat and cholesterol. Lecithin itself helps to bind fats and cholesterol to water so that they can pass through the body rather than cause a potentially harmful buildup in the heart or liver. Lecithin breaks up the bad cholesterol in our blood and prevents sediments of fats, and so lowers blood pressure and the chances of a heart attack (Melvin et l., 1963; Deuticke et al., 1981; Mathur et al., 1996; Rittes, 2001; Rotunda et al., 2004; Hasengschwandtner, 2005).

The present work was focused on the application of statistical methods for optimization of enzymatic milk fat splitting process using soybean lecithin. Various effect of process parameters like initial butter, initial enzyme concentration, temperature and process time were studied and optimized using 3-level central composite design (CCD). CCD is a type of response surface methodology (RSM) follows the following steps (a) experimental observation of percentage of fat splitting using CCD (b) establishing a mathematical model expressing the relationship between the process parameters factors and percentage fat splitting (c) prediction of optimum values of process parameters for maximum percentage fat splitting and (d) experimental verification of the model predicted conditions.

MATERIALS AND METHODS

Milk fat

Milk fat (milk butter) was purchased from local market at Chennai in India was used in this study. The composition of the milk fat was analyzed using gas chromatography. It was found that the fresh milk butter consist of saturated fat 67.35% (w/w), unsaturated fat 32.61% (w/w) and moisture content 0.04% (w/w).

Soybean lecithin

The soybean lecithin used in this study was purchased from Sigma-Aldrich Corporation, Bangalore, India. Soy-bean lecithin solution of 2, 4 and 6% (w/v) was prepared and 5 ml of the solution was used for fat splitting studies.

Response surface methodology

The effect of various process parameters namely initial milk butter, initial enzyme concentration, process time and temperature on enzymatic milk fat splitting process was studied and optimized using 3-level central composite design (CCD). Response surface methodology (RSM) is a statistical technique, based on the fundamental principles of statistics, randomization, replication and, duplication, which simplifies the optimization by studying the mutual interactions among the variables over a range of values in a statistically valid manner. It is an efficient statistical technique for optimization of multiple variables in order to predict the best performance conditions with a minimum number of experiments. CCD is one of the response surface methodologies usually utilized to obtain data that fits a full second-order poly-nomial model. The graphical representation of the model equation results in response surface plots that represent the individual and interactive effects of test variables on the response. The variables are coded to lie "±1" for factorial points and "0" for the center points. The CCD in actual unit of the 4 variables was (Table 1) developed using Minitab15 software. The experimental result was used to find the optimum level of these variables. Effects of these 4 variables on percentage of fat splitting are fit to the second order polynomial model according to Equation (1):

$$Y = b_0 + \sum b_i X_i + \sum b_{ii} X_i^2 + \sum b_{ij} X_i X_j \quad (1)$$

where Y is the response variable to be modeled, X_i and X_j are independent variables in coded units and b_i, b_{ii}, b_{ij} are the measures of the X_i, X_j, X_i^2 and X_iX_j of linear, quadratic and interaction effects respectively. For statistical calculations, the variables were coded according to Equation (2):

$$X_i = (x_i - x_0)/(\Delta x_i) \quad (2)$$

where, X_i is the independent variable in coded unit, x_i is independent variable real value, x_0 is independent variable real value on the centre point and Δx_i is the step change value (Rahulan et al., 2009; Baskar et al., 2009; Rai and Mukherjee, 2010).

The second order polynomial equation was maximized by a constraint search procedure using the MINITAB software 15 to obtain the optimal levels of the independent variables and the predicted maximum percentage fat splitting.

Estimation of total fat content

The total fat content of the milk buffer before and after lecithin treatment was estimated by loss in weight of milk butter (Cowan, 1985).

Estimation of fatty acid content

About 0.25 g of sample was transferred into a 250 ml ground-necked round bottom flask with reflux condenser. 10 ml of 0.5 N sodium hydroxide in methanol and the boiling chip was added. The

Table 1. 3-level CCD in actual unit of the variables on percentage fat splitting.

Run order	Initial milk butter (x_1), (%)	Lecithin concentration (x_2), (%)	Temperature (x_3), (°C)	Digestion time (x_4), (min)	Percentage fat splitting	
					Experimental	Predicted
1	4	6	35	50	1.98	2.69
2	4	2	45	100	2.89	3.93
3	4	2	35	100	2.21	1.85
4	8	2	35	50	1.73	2.02
5	6	6	40	75	6.78	6.20
6	4	6	45	100	4.97	4.32
7	6	2	40	75	3.76	4.98
8	4	4	40	75	4.59	5.18
9	6	4	40	75	7.12	6.90
10	6	4	40	100	7.36	7.36
11	4	2	45	50	3.81	3.19
12	8	6	45	100	6.23	7.24
13	6	4	40	75	7.15	6.90
14	4	6	35	100	2.39	2.21
15	6	4	40	75	7.18	6.90
16	4	2	35	50	2.85	2.02
17	6	4	40	75	7.23	6.90
18	8	6	35	50	4.92	4.07
19	6	4	40	50	6.08	6.72
20	8	6	45	50	5.79	5.78
21	8	2	45	100	6.56	5.48
22	4	2	45	50	3.35	3.71
23	6	4	40	75	7.17	6.90
24	6	4	45	75	7.21	6.85
25	8	2	35	100	2.97	2.89
26	4	6	45	50	3.64	3.90
27	6	4	40	75	7.24	6.90
28	8	4	40	75	6.59	6.64
29	6	4	40	75	7.17	6.90
30	6	4	35	75	3.96	4.96
31	8	6	35	100	4.37	4.62

solution was boiled for 15 min. The condenser was removed after reflux stops. Two drops of phenolphthalein was added to the flask. 1 N sulphuric acid was added until the solution becomes colorless and 1 ml in excess. The content was extracted with pet ether and evaporated to dryness. 20 ml of sulphuric acid in methanol was added and the content was boiled for 20 min with condenser. Then the flask was cooled in running water. The content was extracted with pet ether thrice and ether layer was washed with water three to four times. The ether layer was passed through anhydrous sodium sulphate and evaporated to dryness. Reconstituted in pet ether and the fatty acids content was analyzed using gas chromatography (BIS, 1976).

RESULTS AND DISCUSSION

Optimization of milk fat splitting process using response surface methodology

The percentage of fat splitting was obtained by conducting experiments based on 3-level central composite design given in Table 1. The student's t-test and fisher's F-test on experimental percentage of fat splitting were performed using MINITAB 15.0 software. The coefficients, t and p values for linear, quadratic and interaction effects of initial milk butter, initial enzyme concentration, process time and temperature on percentage of fat splitting were given in the Table 2, at 95% significance level. The p-values in student's t-test are used as a tool to check the significance of each of the coefficients, which in turn may indicate the pattern of the interactions between the variable. The smaller the p-value, more significant is the corresponding coefficient. It was observed that the overall effect of the variables has significantly increased the percentage fat split ($p < 0.001$).

The positive coefficients and low p-values of initial milk butter (X_1), initial enzyme concentration (X_2) and

Table 2. Estimated regression coefficients for optimization of percentage fat splitting.

Variable	Estimated coefficient	t-value	p-value
Constant	6.902	28.357	<0.001
X_1	0.732	3.786	0.002
X_2	0.607	3.142	0.006
X_3	0.948	4.903	<0.001
X_4	0.322	1.666	0.115
X_1*X_1	-0.989	-1.942	0.070
X_2*X_2	-1.309	-2.570	0.021
X_3*X_3	-0.994	-1.952	0.069
X_4*X_4	0.140	0.276	0.786
X_1*X_2	0.342	1.670	0.114
X_1*X_3	0.128	0.628	0.539
X_1*X_4	0.260	1.267	0.223
X_2*X_3	0.007	0.037	0.971
X_2*X_4	-0.078	-0.384	0.706
X_3*X_4	0.225	1.097	0.289

Table 3. Analysis of variance for optimization of percentage fat splitting.

Source	Degree of freedom (DF)	Sum of square (SS)	Mean square (MS)	F-value	p-value
Regression	14	101.013	7.215	10.72	<0.001
Linear	4	34.356	8.589	12.76	<0.001
Square	4	62.522	15.630	23.21	<0.001
Interaction	6	4.133	0.689	1.02	0.445
Residual error	16	10.773	0.673		
Total	30	111.786			

temperature (X_3) indicates the significant increase in percentage fat split due to their individual effect with p = 0.002, p = 0.006 and p < 0.001, respectively. The individual effect of process time (X_4) has no significant influence (p = 0.115) on percentage fat split. The negative coefficient of quadratic effect of initial enzyme concentration (p = 0.021) has significantly decreased percentage fat split. Whereas the other variables like milk butter (p = 0.070), temperature (p = 0.069) and process time (p = 0.786) has no significant influence on percentage fat split. It was found from student's t-test in Table 2 that there were no interaction effects (p > 0.05) between the variables studied on percentage fat split. The results of student's t-test imply that the independent variables selected for optimization have greater influence on enzymatic milk fat splitting process using soybean lecithin.

Analysis of variance (ANOVA) was used to test the significance and adequacy of the second order polynomial model. The ANOVA of the model shown in Equation (3) is given in Table 3 at 95% confidence level. Generally, the calculated F-value should be several times greater than the tabulated F-value if the model is a good prediction of the experimental results and the estimated effects are real. In this study, the ANOVA of the polynomial model demonstrates that it is highly significant, evident from the calculated F-value (F-model = 10.72) and probability value (p < 0.001). It is also evident from ANOVA that the linear (p < 0.001) and quadratic effect (p < 0.001) of the model has greater influence on percentage fat split and no significant influence (p = 0.445) was observed due to the interaction effect of the variables:

$$Y = 6.903 + 0.732X_1 + 0.607X_2 + 0.948X_3 + 0.322X_4 - 0.989X_1^2 - 1.309X_2^2 - 0.994X_3^2 + 0.141X_4^2 + 0.342X_1X_2 + 0.128X_1X_3 + 0.260X_1X_4 + 0.007X_2X_3 - 0.078X_2X_4 + 0.225X_3X_4 \quad (3)$$

where Y is percentage fat splitting and X_1, X_2, X_3 and X_4 are in coded unit of the variables. The correlation measures for testing the goodness of fit of the second order polynomial model is the coefficient of determination

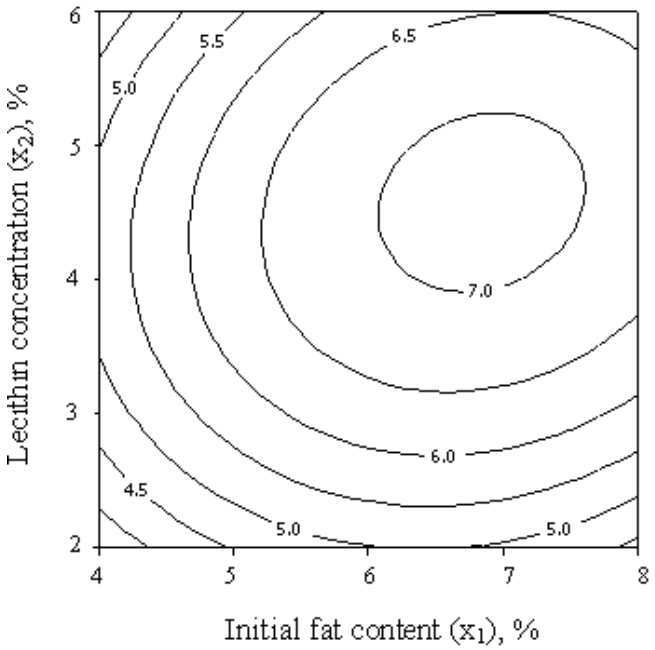

Figure 1. Response contour plot showing the interaction effect of initial milk butter and lecithin concentration on percentage fat splitting (Hold values: temperature, 40°C and digestion time, 75 min).

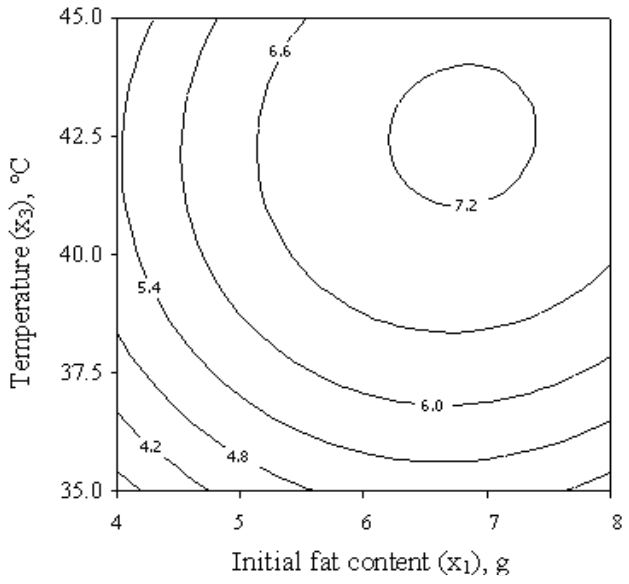

Figure 2. Response contour plot showing the interaction effect of initial milk butter and temperature on percentage fat splitting (Hold values: Lecithin concentration, 4% and digestion time, 75 min).

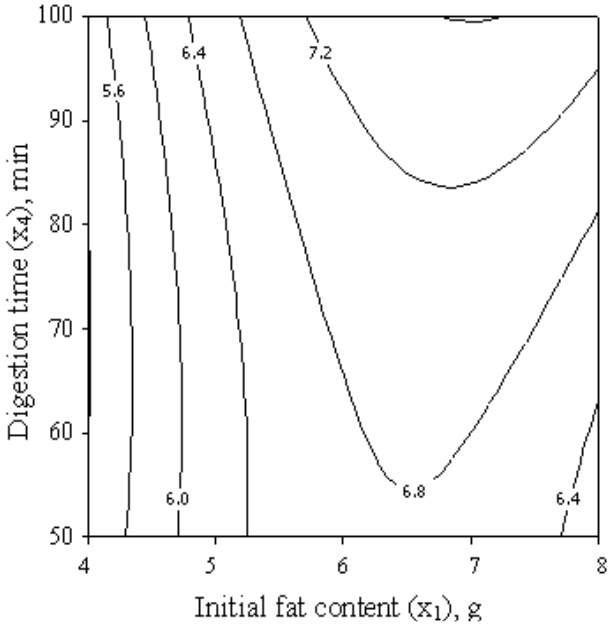

Figure 3. Response contour plot showing the interaction effect of initial milk butter and digestion time on percentage fat splitting (Hold values: Lecithin concentration, 4% and temperature, 40°C).

(R^2). When R^2 is closer to 1, correlation is better between the experimental values and the values predicted by the second order polynomial model. The high R^2 value implies high degree of correlation between the observed and predicted values. The adjusted determination coefficient (Adj R^2) corrects the R^2 value for the sample size and the number of terms in the model. There are many terms in the model and the sample size is not very large, therefore the adjusted R^2 noticeably smaller than the determination coefficient R^2. The adjusted R^2 in this study was 0.819, which is close to the R^2 (0.904) value. The polynomial model given in Eqution (3) predicts very well the experimental percentage fat splitting. Hence the model represents very well the effect of initial milk butter, initial enzyme concentration, process time and temperature on enzymatic milk fat splitting process using statistical optimization.

The graphical representations of the interaction effect of the variables called the contour plots were developed using MINITAB 15.0 software and interaction between any two variables was studied on percentage fat splitting, keeping other two variables constant at their middle values. The shape of the response surface plots, elliptical or circular, indicates the interactions between the variables are significant or not. The circular shape of the contour plots between initial butter and initial enzyme concentration (Figure 1), initial butter and temperature (Figure 2) and initial enzyme concentration and temperature (Figure 4) indicates that there was significant interaction effect between these variable set on percentage of enzymatic milk fat splitting. Whereas the elliptical shape of the contour plots between initial butter and digestion time (Figure 3), initial enzyme concentration and digestion time (Figure 5) and temperature and digestion time (Figure 6) indicates that there was no

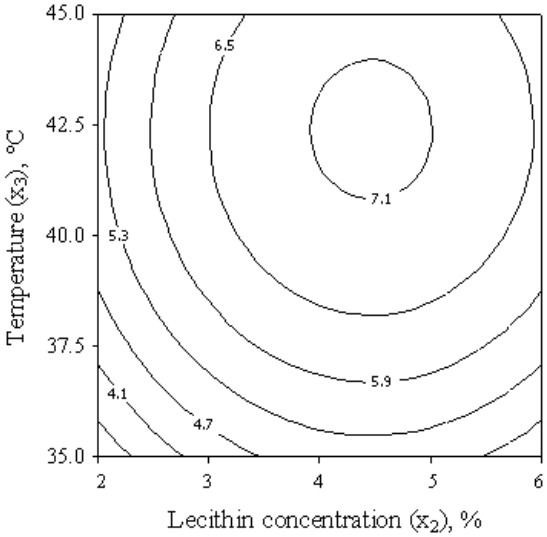

Figure 4. Response contour plot shows the interaction effect of lecithin concentration and temperature on percentage fat splitting (Hold values: Initial milk butter, 6% (w/v) and digestion time, 75 min).

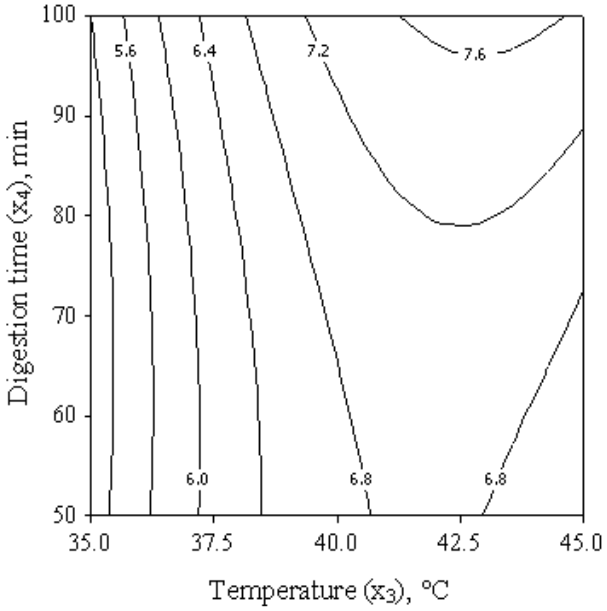

Figure 6. Response contour plot shows the interaction effect of temperature and digestion time on percentage fat splitting (Hold values: Initial milk butter 6% (w/v) and lecithin concentration, 4%).

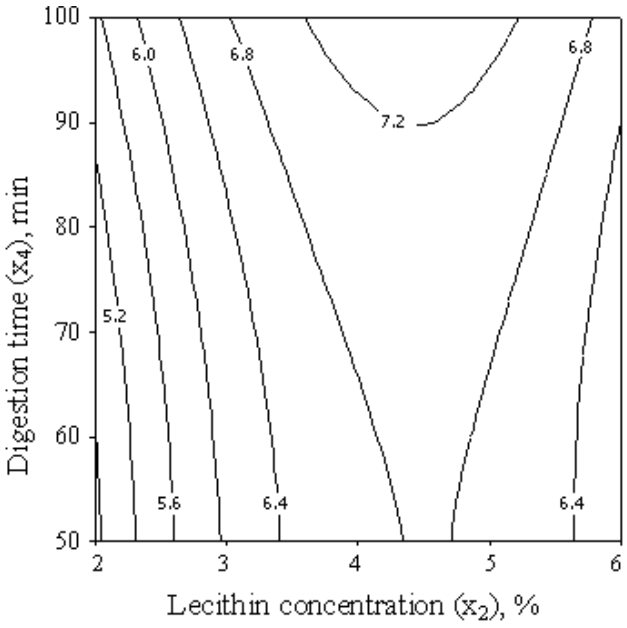

Figure 5. Response contour plot showing the interaction effect of lecithin concentration and digestion time on percentage fat splitting (Hold values: Initial milk butter, 6% (w/v) and temperature, 40°C.

significant interaction effect between these variable set on percentage of enzymatic milk fat splitting. The polynomial model in Equation (3) was solved by response optimizer in MINITAB 15.0 software to obtain global optimum value of the variables for maximum percentage of fat splitting.

Figure 7, the normal probability plot of the residuals is an important diagnostic tool to detect and explain the systematic departures from the assumptions. The residual was plotted against normal distribution of the model and it is approximately linear for percentage of fat splitting. That the errors are normally distributed and are independent of each other and that the error variances are homogenous. An excellent normal distribution confirmed the independence of the residuals. This indicates that the model was well fitted with the experimental results. As the residuals from the fitted model are normally distributed, all the major assumptions of the model have been validated. The residual plot in Figure 8 shows equal scatter of the residual data above and below the x-axis indicates that the variance was independent of the percentage fat splitting, thus supporting the adequacy of the model fit. The predicted optimum conditions for the maximum percentage fat splitting was initial butter, 6% (w/v), initial enzyme concentration, 4% (w/v), temperature, 40°C and digestion time 89.99 min for the maximum percentage of fat splitting 7.15% (w/w). The confirmation experiment was conducted at the optimum conditions for validating the model under experimental conditions. The experimental maximum percentage fat splitting of 7.38% (w/w) was obtained at the optimum conditions. The experimental and predicted values of percentage of fat splitting showed a good agreement with one another; with a high degree of accuracy of the model indicating that the central composite design of response surface method was effective in optimization of enzymatic milk fat splitting

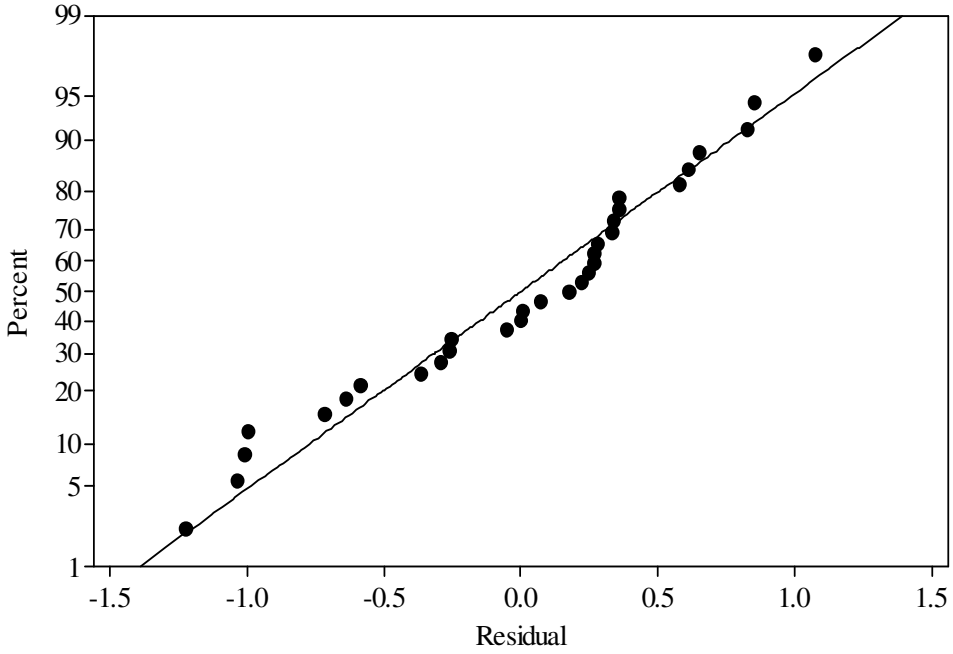

Figure 7. The normal probability plot on percentage fat splitting.

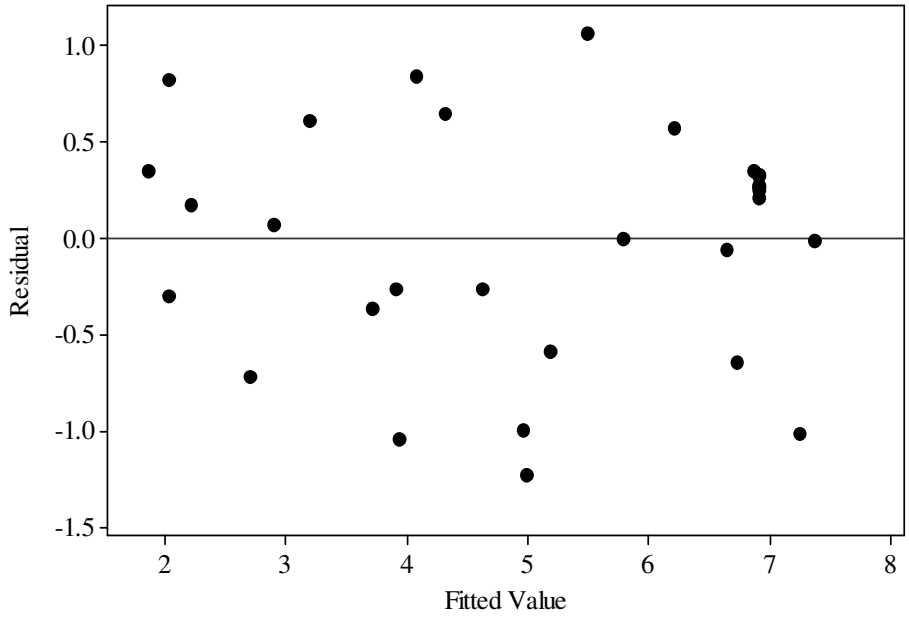

Figure 8. Residual distribution plot on percentage fat splitting.

process. The percentage of fat splitting obtained in the present work was 17.89% higher than obtained by classical method (Panwal, 2010).

Conclusion

The response surface methodology was effectively applied for optimization of enzymatic milk fat splitting by soybean lecithin. The high value of coefficient of determination (R^2 = 0.904) of the second order polynomial model of response surface methodology showed the good prediction of the experimental percentage of fat splitting. The enhanced percentage of fat splitting of 7.38% (w/w) was obtained at initial butter, 0.6% (w/v), initial enzyme concentration, 4% (w/v), temperature, 40°C and digestion

time, 89.99 min. The percentage fat splitting using soybean lecithin was found high at mild operating conditions.

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REFERENCES

Barnebey HL, Brown AC (1948). Continuous fat splitting plants using the colgate-emery process. J. Am. Oil Chem. Soc., 25: 95-99.

Baskar G, Dharmendira Kumar M, Anand Prabu A, Renganathan S, Yoo CK (2009). Optimization of carbon and nitrogen sources for L-asparaginase production by *Enterobacter aerogenes* using response surface methodology, Chem. Biochem. Eng. Q., 23: 393-397.

BIS IS 548-3:1976 (R2006). Methods of sampling and test for oils and fats - part 3: Analysis by gas liquid chromatography**.**

Brook JG, Linn S, Aviram M (1986). Dietary soya lecithin decreases plasma triglyceride levels and inhibits collagen and ADP induced platelet aggregation. Biochem. Med. Metab. Biol., 35: 31-39.

Bruha R, Marecek Z (2000). Essential phospholipids in the treatment of hepatic encephalopathy, Vnitr. Lek., 46: 199-204.

Cowan PE (1955). Weight of dried marrow as an indicator of femur fat on brushtail possums *Trichosurus vulpecula*, NZ J. Zool., 12: 349-352.

David K (1995). Fatty acids, triglyceride structure and lipid metabolism. J. Nutr. Biochem., 6: 172-178.

Deuticke B, Grunze M, Forst B, Luetkemeier P (1981). Influence of enzymatic phospholipid cleavage on the permeability of the erythrocyte membrane: III. Discrimination between the causal role of split products and of lecithin removal, J. Membr. Biol., 59(1): 45-55.

Golay A, Bobbioni E (1997). The role of dietary fat in obesity, Int. J. Obes., 21 (Suppl. 3): S2-11.

Hasengschwandtner F (2005). Phosphatidylcholine treatment to induce lipolysis, J. Cosmet. Dermatol., 4: 308-313.

Mathur SN, Born E, Murthy S, Field FJ (1996). Phosphatidylcholine increases the secretion of triacylglycerol-rich lipoproteins by CaCo-2 cells. Biochem. J., 314: 569-575.

Melvin JS, Bianca R, Mordecai T, Zygmunt L (1963). Lipid metabolism and arteriosclerotic heart disease in Israelis of Bedouin, Yemenite and European origin, Am. J. Cardiol., 12: 157-168.

Muckerheide VJ. (1952). Fat splitting and distillation. J. Am. Oil Chem. Soc., 29: 490-495.

Panwal JH, Viruthagiri T, Baskar G (2010). Optimization and thermodynamics studies on enzymatic milk fat splitting process using soybean lecithin, Adv. J. Food Sci. Technol., 2: 250-255.

Rahulan R, Nampoothiri KM, Szakacs G, Nagy V, Pandey A (2009). Statistical optimization of L-leucine amino peptidase produc-tion from *Streptomyces gedanensis* IFO 13427 under submerged production from *Streptomyces gedanensis* IFO 13427 under submerged fermentation using response surface methodology. Biochem. Eng. J., 43: 64-71.

Rai SK, Mukherjee AK (2010). Statistical optimization of production, purification and industrial application of a laundry detergent and organic solvent-stable subtilisin-like serine protease (Alzwiprase) from *Bacillus subtilis* DM-04, Biochem. Eng. J., 48: 173-180.

Rittes, PG (2001). The use of phosphatidylcholine for correction of lower lid bulging due to prominent fat pads. Dermatol. Surg., 27: 391-392.

Romieu I, Lajous M (2009). The role of obesity, physical activity and dietary factors on the risk for breast cancer: Mexican experience, Salud Publica de mexico, 51(Suppl. 2): S172-180.

Rotunda AM, Suzuki H, Moy RL, Kolodney MS (2004). Detergent effects of sodium deoxycholate are a major feature of an injectable phosphatidylcholine formulation used for localized fat dissolution, Dermatol. Surg., 30: 1001-1008.

Schwartz MJ, Lewitus Z (1962). A study of lipid metabolism and arteriosclerotic heart disease in Israelis of European and Yemenite origin, J. Atheroscler. Res., 2: 68-78.

Wallnoefer H, Hanusch M (1973). Essential phospholipids in the treatment of hepatic disease. Med. Monatsschrift., 27: 131-136.