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Studies on Various Parameters Involved in Conjugation of Starch with Lysine for Excellent Emulsification Properties Using Response Surface Methodology

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Abstract**—**The process parameters, starch-water ratio (A, (w/v) %), pH of suspension (B), Temperature(C, °C) and Time (D, hrs.)., were optimized for the preparation of starch-lysine conjugate and studying their effect on stability of emulsions by calculating emulsion stability index using response surface methodology. The optimized conditions are pH 9.0, temperature 60^{o}C, reaction time 6 hrs, starch:water ratio 1:2.5, having emulsion stability index was 0.72.

Keywords**—**Emulsion stability index, pH of suspension, Starch-water ratio, Temperature, Time.

I. Introduction

THE various properties of the starch can be utilized in food and industrial applications such as thickener, water retention agent, colloidal stabilizer, gelling agent, bulking agent and adhesive. Its applications in various food and industrial sector are mainly due to its gelatinization and retrogradation behavior. Various amino acids such as lysine, polylysine, monosodium glutamate, glycine, alanine, leucine, epsilon – aminocaproic acid, cysteine and glutamine can be used for modification of gelatinization behavior of potato starch [1], [4].

Modified starch is generally having variety of applications in food industry. It can be used for substituting fat [5], for improving texture of food products, nutritional substitute for fast food products [6], [7], for stability to high temperatures, low pH and shear degradation during food processing [8]-[10], for aroma encapsulation [11], [12] etc.

For using modified starches in food applications, it is important to achieve functional changes in starch so as to improve their applications in emulsions also. Gum arabic is commonly used to stabilize beverage emulsions which can be replaced by modified starches.

C☆EmCap (a product of Cargill) modified food starch is a cost-effective alternative to conventional emulsifiers, with emulsifying and flavor protection properties in one neat product. It is also having the properties to retain expensive flavor oils and essences, encapsulate and protect them from breakdown, and emulsifies oil and water solutions in beverages, liquid and dry clouds, even salad dressings. Flavor emulsions and finished beverages show no oil separation or ringing after 6 months at room temperature [13].

In the following study, starch is conjugated with lysine by using maillard reaction, with variable pH, temperature, water-starch ratio and reaction time.

II. Materials And Methods

A. Materials

Potato starch was procured from Ranbaxy Fine Chemicals Ltd. Lysine was procured from Himedia labs ltd.

B. Methods

Potato starch was conjugated with 5% amino acid (lysine) with variable starch and water ratio. Variable pH of the suspension adjusted using 3M NaOH or 3M HCL.

Reaction proceeded at variable temperatures and at variable reaction time. After neutralization, the conjugate was retrieved using centrifugation and thereafter 4-5 times washing with double distilled water. The conjugate formed was further washed with ethanol and oven dried at 40^{0}C. The emulsion stability index of the dried powder was measured accordingly.

C. Optimization of Process Parameters Using Response Surface Methodology

Response surface methodology is basically a sequential process leading the experiments more rapidly and in a better efficiently manner in the direction towards improvement of the optimum values. Starch/water ratio, pH of suspension, temperature and reaction time were chosen for optimization of major variables responsible in conjugate preparation. A central composite design (CCD) for four independent variables with 24 plus 6 centre points leading to a total of 30 experiments was used. The experiments were carried out at least in triplicate, which was necessary to estimate the variability of the measurements i.e. for the reproducibility of the experiments. The experiments were designed using the software Design Expert Version 8.0.4 (State Ease, Minneapolis, MN).

Table I shows different levels of the studied variables i.e. starch-water ratio (A, (w/v) %), pH of suspension (B), Temperature(C, °C) of reaction and Time (D, hrs.) of reaction. Emulsion stability index was taken as a dependant variable (or) Response.

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TABLE I
VALUE FOR CODED AND ACTUAL FACTORS FOR THE CENTRAL COMPOSITE DESIGN

Codes	LEVELS					
	-2	-1	0	1	2	Δx
Starch/water ratio	1:1.5	1:2.5	1:3.5	1:4.5	1:5.5	1
pH of suspension	6	7	8	9	10	1
Reaction time (hrs.)	3	4	5	6	7	1
Temperature (^{0}C)	30 ^{0}C	40 ^{0}C	50 ^{0}C	60 ^{0}C	70 ^{0}C	10

The central values (zero level) considered in the design are starch-water ratio 1:3.5, pH of suspension 8, reaction time 5 hrs. and temperature 50^{0}C. A regression equation was derived considering 1st and 2nd order models based on the coded values.

1. Validating the Quadratic Model Suggested by RSM

Two experiments are performed to validate the fitness of the suggested model. The first one consisted starch-water ratio 1:3.5, pH of suspension 10, reaction time 5 hrs. and temperature 50^0 C. The second one consisted starch-water ratio (1:4.5), pH of suspension 9, reaction time 4 hrs. and temperature 40^0 C was checked for emulsion stability index.

D. Estimation of Emulsion Stability Index

The experiments are performed in 100 ml shake flask under controlled conditions. The emulsion stability index of the conjugate was estimated according to the method of Pearce and Kinsella[14].

A sample of liquid emulsion was transferred to a 10 ml measuring cylinder, which was then capped and stored for 24 h. The volume of oil separated from the emulsion in the cylinder was determined. Results are reported as emulsion stability index (ESI) with a range of possible results from 0 to 1.

$$ESI = 1 - \frac{\text{Total volume of oil in emulsion}}{\text{Total volume of separated oil}}$$

A value of 0 represents poor emulsion stability while a value of 1 represents high emulsion stability.

III. RESULTS AND DISCUSSIONS

The central composite design suggested a quadratic model based on the given set of experimental results. Table II shows the experimental and predicted values of emulsion stability index for the total set of experiments.

TABLEII
CENTRAL COMPOSITE DESIGN OF SELECTED VARIABLES

Std. run	Factor A	Factor B	Factor C	Factor D	Emulsion stability Index		
	Starch/water ratio	pH of suspension	Reaction time (hrs.)	Temperature (^{0}C)	Experimental[a]	Predicted	Residual
1	-1.00	-1.00	-1.00	-1.00	0.013	0.047	-0.034
2	1.00	-1.00	-1.00	-1.00	0.56	0.52	0.044
3	-1.00	1.00	-1.00	-1.00	0.36	0.29	0.067
4	-1.00	1.00	-1.00	-1.00	0.64	0.64	0.003083
5	-1.00	-1.00	1.00	-1.00	0.001	-0.055	0.056
6	1.00	-1.00	1.00	-1.00	0.18	0.18	0.001417
7	-1.00	1.00	1.00	-1.00	0.02	0.060	-0.040
8	1.00	1.00	1.00	-1.00	0.18	0.17	0.009833
9	-1.00	-1.00	-1.00	1.00	0.02	-0.074	0.094
10	1.00	-1.00	-1.00	1.00	0.25	0.29	-0.035
11	-1.00	1.00	-1.00	1.00	0.2	0.28	-0.077
12	1.00	1.00	-1.00	1.00	0.56	0.51	0.048
13	-1.00	-1.00	1.00	1.00	0.36	0.44	-0.079
14	1.00	-1.00	1.00	1.00	0.6	0.56	0.036
15	-1.00	1.00	1.00	1.00	0.72	0.66	0.060
16	2.00	0.00	0.00	0.00	0.62	0.66	-0.041
17	-2.00	0.00	0.00	0.00	0.08	0.090	0.009583
18	2.00	0.00	0.00	0.00	0.54	0.56	-0.019
19	0.00	-2.00	0.00	0.00	0.28	0.31	-0.028
20	0.00	2.00	0.00	0.00	0.65	0.65	-0.0005833
21	0.00	0.00	-2.00	0.00	0.001	0.042	-0.041
22	0.00	0.00	2.00	0.00	0.1	0.088	0.012
23	0.00	0.00	0.00	-2.00	0.2	0.24	-0.040
24	0.00	0.00	0.00	2.00	0.62	0.61	0.011
25	0.00	0.00	0.00	0.00	0.082	0.080	0.002167
26	0.00	0.00	0.00	0.00	0.086	0.080	0.006167
27	0.00	0.00	0.00	0.00	0.077	0.080	-0.002833
28	0.00	0.00	0.00	0.00	0.05	0.080	-0.030
29	0.00	0.00	0.00	0.00	0.1	0.080	0.020
30	0.00	0.00	0.00	0.00	0.084	0.080	0.004167

[a] A= Starch/water ratio B= pH of suspension C=Reaction time (hrs.) D= Temperature (^{0}C), [b] Degree of Freedom ,[c] # - not significant; * $p < 0.05$, $R^2 = 0.97$

R^2 was found to be 0.9714 for Emulsion stability index i.e. 97.14 % of the experimental data was fitting according to the data predicted by the model and only 2.86 % variations will not be explained by the model. The value Adj. R^2 is 0.9447 showing high significance of the model. High value of deviation between of experimental and predicted values was observed i.e. CV of 20.99. In the current context of work, the ratio is 18.053, indicates an adequate signal. The mathematical expression of the correlation between the emulsion stability index to the variables is shown below.

Emulsion stability = + 0.079833 + 0.11733 × Starch/water ratio + 0.085667 × pH of suspension + 0.011500 × Reaction Time + 0.092333 × Temperature - 0.031000 × Starch/water ratio × pH of suspension - 0.058625 × Starch/water ratio × Reaction Time - 0.027250 × Starch/water ratio × Temperature - 0.032375 × pH of suspension × Reaction Time + 0.026500 × pH of suspension × Temperature + 0.15388 × Reaction Time × Temperature + 0.061104 × Starch/water ratio^2 + 0.099854 × pH of suspension^2 - 3.77083E-003 × Reaction Time^2 + 0.086104 × Temperature^2

Table III shows the analysis of variance of the variables. For model to be significant, the values of Prob>F should be less than 0.05. In the present work, the model terms A, B, D, AB, AC, BC, CD, A^2, B^2, D^2 are significant.

TABLE III
ANALYSIS OF VARIANCE (ANOVA) FOR THE MODEL

Source	Coefficient factor	F value	P-value Prob> F
Model	0.080	36.39	< 0.0001
A	0.12	*99.60*	*< 0.0001*
B	0.086	*53.09*	*< 0.0001*
C	0.011	*0.96*	*0.3435*
D	0.092	*61.68*	*< 0.0001*
AB	-0.031	*4.63*	*0.0480*
AC	-0.059	*16.58*	*0.0010*
AD	-0.027	*3.58*	*0.0779*
BC	-0.032	*5.06*	*0.0400*
BD	0.027	*3.39*	*0.0856*
CD	0.15	*114.20*	*< 0.0001*
A^2	0.061	*30.87*	*< 0.0001*
B^2	0.100	*82.44*	*< 0.0001*
C^2	-3.771E-003	*0.12*	*0.7364*
D^2	0.086	*61.30*	*< 0.0001*
Residual			
Lack of Fit		*17.73*	*0.0027*
Pure Error			
Cor Total			

A= Starch/water ratio; B = pH of suspension; C=Reaction time (hrs.); D= Temperature (^{0}C)

Figs. 1-4 showing the contour plots of the interaction between the variables and the optimum level of each variable on emulsion stability index. Fig. 1 illustrates that uptil pH 8, the emulsion stability index starts increasing and thereafter it started decreasing. Fig. 2 shows uptil starch-water ratio of 1:2.5 hrs., the emulsion stability index starts increasing and thereafter it started decreasing. Fig. 3 shows until pH of 7.5 hrs., the emulsion stability index goes on increasing and thereafter it started decreasing. Although there was not much impact on the effect of reaction time on the emulsion stability index, uptil the reaction time of 6 hrs., the emulsion stability index increases and after that it started decreasing. Also the studies of the contour plot shows that the optimum range of the process parameters are pH 7-9, temperature 40-60^{o}C, reaction time 5-6 hrs., starch:water ratio1:1.5-1:2.5.

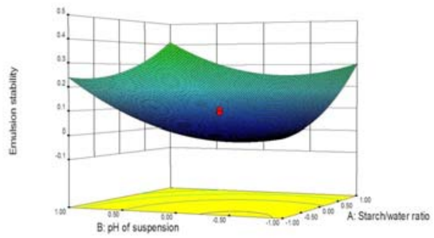

Fig. 1 The 3D plot showing the effects of pH, starch water ratio and their mutual interaction on emulsion stability index

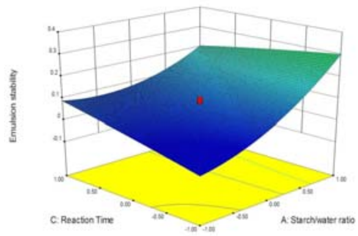

Fig. 2 The 3D plot showing the effects of reaction time, starch water ratio and their mutual interaction on emulsion stability index

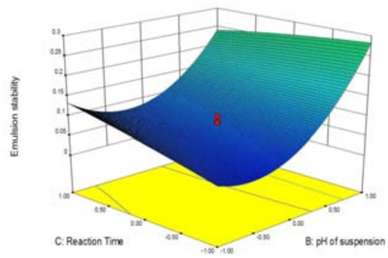

Fig. 3 The 3D plot showing the effects of reaction time, pH and their mutual interaction on emulsion stability index

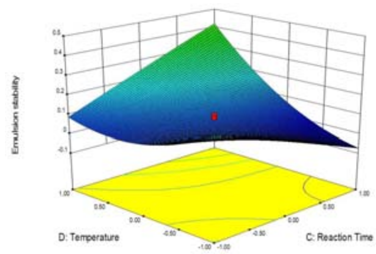

Fig. 4 The 3D plot showing the effects of temperature, reaction time and their mutual interaction on emulsion stability index

The optimized process parameters obtained are pH 9, temperature 60^{o}C, reaction time 6 hrs., starch:water ratio 1:2.5. Also the optimum results obtained for the parameters (pH, temperature, reaction time and starch:water ratio) are validated. The maximum emulsion stability was found to be 0.66 which was in near range to the predicted model.

IV. Conclusions

Response surface methodology may be used for the process parameter optimization involved in the preparation of potato starch-lysine conjugate for its application in emulsion stability. The model developed for CCD was found to be significant. The optimum values are found to be pH 9, temperature 60^{o}C, reaction time 6 hrs., starch:water ratio1:2.5. Also it was found that the optimum results will be having better emulsion stability index (0.72-near to 1) and therefore can be used as the emulsifier for the stability of the emulsions.

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