Optimization of Preparation Process of Tapioca Starch-Lipid Complex by Response Surface Methodology

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Abstract Starch-lipid complex is endow with higher resistance to digestive enzymes than amylase alone, and the complex can combine more hydrophobic substances. In this work, thermostable α-amylase was involved to hydrolyze the starch as pretreatment. Then response surface methodology was used to explore the effects of lipid addition amount, stirring time and water bath temperature on complexing index (CI) of starch and soybean lecithin and established a mathematic model of optimal complex conditions. The results showed that the optimal lipid addition amount, stirring time, bath temperature were 3.5 %, 70 min and 89 ℃. Under the optimal conditions, the experimental CI was 51.4429, which was close to the predicted CI of 51.4763. The obtained regression model was fitting in good condition and had met the design requirements.

Keywords Complexation, Tapioca starch, Soybean lecithin, Response surface methodology, optimization.

Introduction

The starch granule is nature's chief way of storing energy over long periods in green plants and is synthesized in a granular form. The two major macromolecular components of starch are amylose and amylopectin of the granule. They are a type of glucan made of glucose connected in two different ways. Amylose is the predominantly linear (1→4)-linked α-glucan. Amylopectin, α-(1→4)-linked α-glucan with α-(1→6) branch points, is the major component of the granule [1]. They can be identified only after separation following solubilization.

In a freshly prepared aqueous solution, amylose is present as a random coil [2]. The random coil conformation, however, is not stable. Amylose tends to form either single-helical (inclusion) complexes with suitable complexing agents [3]. Lipids can form stable complexes with both amylose and long branch chains of amylopectin [4]. In a single-helical complex, the linear portion of the starch molecule has its hydrophobic side of the molecule facing the cavity of the helix and interacting with the non-polar moiety of the complexing agent. Molecules with small cross-sections, such as fatty acids and glycerol monostearate [5-6], complex with amylose to form helices of six glucose units per turn.

Starch and lipid V-type complex (RS5), a type of resistant starch, is equipped with higher resistance to digestive enzymes than amylase alone [7-9]. The formation process of RS5 is very complex, and its structure depends on a lot of factors, such as the polymerization and branching degree of starch, concentration, reaction temperature and the structure of lipids, etc [5, 10-11]. The hydrophobicity, degradation and complexation properties can be adjusted to meet the requirements of products, due to the interaction of starch and lipids. The starch and lipids complex with enhanced properties can be applied to starch-based additives or condiments to improve their texture and structure stability, such as reducing viscosity, strengthening the freeze-thaw stability and delaying retrogradation. On account of the lipids components, the complex can combine more hydrophobic substances like oil, flavour constitutes of food, antioxidants, drugs and bond materials [12].

Tapioca starch was used in study on inclusion complex with ligands. The main reason for this is that because tapioca starch contains a low amount of internal lipid (<0.1%) which interferes with the formation of the complex [13-14].

Starch-lipid complex has a unique application advantage, thus the optimization of preparation process of starch-lipid complex by response surface methodology was studied in this paper. In order to optimize the reaction, a statistical method, response surface methodology (RSM) is often introduced to overcome the limitations of

classic empirical methods and proves to be powerful tool for the optimization of the target value [15]. In this study, using the RSM method based on the Box-Benhnken (BBD) technique and by definition of three effective factors including lipid addition amount, stirring time and bath temperature, the main effects of factors and their interactions on the response of complexing index are studied and optimized. Moreover, for response the appropriate fitted models are presented.

Materials and methods
Materials
Tapioca Starch was from Daklak Agricultural Materials and Food Joint-stock Company (Daklak, Vietnam). Soybean lecithin kindly donated by Zhengzhou four-dimensional phospholipid technology Co. Ltd. Thermostable α-amylase was provided by Novozymes (Tian, China). Ethanol and Acetic acid glacial were of analytic grade and purchased from Tianjing Tianli Chemical Company (Tianjing, China), Iodine and Potassium iodide were from Kermel Chemical Company (Tianjin, China). All other reagents were of analytic grade and obtained from local sources.

Starch-lipid complexation
Tapioca starch (120g) was weighed into 500ml round-bottomed flask and distilled water was added. Thermostable α-amylasewith a volume of 5 ul (140 U) was added to starch suspensions (30% w/v) prepared above, and heated in water bath for a given time, while continuously agitating with the use of a mechanical stirrer, at 500 rpm. The time for hydrolysis lasted for 5 min when the temperature of starch suspensions was up to 72 ℃，then destroyed enzyme using HCl solution followed by NaOH neutralization. Starch past with different amount of lipids was equilibrated in water bath and agitated for a certain time. After the processes were done, paste was cooled at room temperature then frozen with liquid nitrogen and dried by vacuum freeze dryer. Finally the dried complex was shattered with the aid of a pulverizer and sifted through mesh.

Complexing index
Dry matter of sample (0.1000g) was accurately weighed in beaker and anhydrous ethanol (1 ml) was added to fully wet sample, the sample was dispersed by NaOH(9 mL, 1 mol/L) in water bath for 10 min followed by adjusting to constant volume(100mL). Then the dispersion(5.00 mL) was measured to volumetric flask(100 ml) contained distilled water(50 ml) and acetic acid solution(1 mL, 1 mol/L) followed by adjusting to constant volume(100mL) using distilled water. The absorbance was measured at 620 nm after adding iodine solution for 10 min. The iodine solution used for analysising was prepared by dissolving potassium iodide(2 g) and Iodine(0.2 g) in 50 ml distilled water. Then the final volume was made to 100 ml using distilled water. Complexing index was calculated from the equation.

$$\text{CI (\%)} = (\text{Absorbance of control} - \text{absorbance of sample}) \times 100 / \text{absorbance of control} \tag{1}$$

Experimental design
Optimization techniques which carried out by determining the influence of one factor at a time on response while others kept at constant level has major disadvantages that are high number of required data and inability to consider interaction effect among different variables. So, for avoiding these problems, response surface methodology was carried out. The objective is to simultaneously optimize the levels of operating variables to obtain the best response.

Based on the result of single factor experiments, the three major factors and their levels, including additive amount of lipid(known by symbol A), stirring time(symbol B), temperature of water bath(symbol C), are designed and a 3-level-3-factor BBD is employed to optimize this complexation. The variables and their coded and uncoded values are presented in **Table 1**.

Table 1: Factors and Levels in response surface design

Effective factor	Symbol	Levels		
		-1	**0**	**1**
Additive amount (%)	A	2	3	4
Time (min)	B	45	60	75
Temperature (℃)	C	85	90	95

Results and discussion
Fitting the model and analysis of experimental data
Based on the BBD design for three independent factors, all experimental runs and results were depicted in **Table 2**. It demonstrates the list of these runs including the trend of factors variations and value of each response. The present research was done for the responses of complexing index which was known by R symbol. Afterwards, according to experimental data of these responses, using software the quadratic polynomial

equation was presented for response that can predict the relationship of response value and the amounts of defined factors. The fitted model on the complexing index of samples can be presented as follows.

$$R = 50.55 + 1.92\,A + 1.38\,B - 0.44\,C + 1.17\,AB - 0.36\,AC$$

$$- 0.77\,BC - 2.99\,A^2 - 1.65B^2 - 2.56\,C^2 \tag{2}$$

where R is complexing index，A, B, and C are response for additive amount of lipid, stirring time, temperature of water bath, respectively.

In order to determine whether or not the quadratic model is significant, it is necessary to conduct ANOVA analysis. The data are presented in **Table 3**, it shows that the quadratic model is statistically significant ($P < 0.0001$) and suitable to describe the reaction of starch and Soya bean lecithin. Additive amount of lipid (A), stirring time (B), temperature of water bath (C) all have a significant effect on complexing index. The interaction terms (AB, BC) and the quadratic terms (A^2, B^2, C^2) are significant. The P-value of 'lack of fit' is 0.6781, which indicates that'lack of fit' is insignificant relative to the pure error.

The "Pred R-Squared" of 0.9382 is in reasonable agreement with the "Adj R-Squared" of 0.9801, the low difference between two ranges can be used as an evidence of conformity of predicted value by model with experimental data. Accordingly, the R-squared (R^2) value is equal to 0.9929 for R model; hence, it can be concluded that the presented model has considerable predictability. **Figure1** indicate the relationship plots of actual data and predicted values by model for complexing index. These plots show the significant matching between predicted values and actual data as a reason of high values of R-squared.

Table 2: Experimental design and results for RSM

Run order	Factors			Responses
	A	B	C	R
1	4	45	90	45.02
2	3	60	90	50.87
3	3	75	95	46.38
4	2	45	90	43.65
5	2	60	85	43.15
6	2	75	90	44.46
7	3	60	90	50.02
8	2	60	95	42.86
9	4	60	85	47.85
10	4	75	90	50.52
11	4	60	95	46.13
12	3	75	85	48.67
13	3	60	90	50.77
14	3	45	85	44.76
15	3	45	95	45.56

Table 3: Variance analysis of the regression model.

| Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value
Model	113.94	9	12.66	77.77	< 0.0001
A	29.64	1	29.64	182.10	< 0.0001
B	15.24	1	15.24	93.59	0.0002
C	1.53	1	1.53	9.41	0.0279
AB	5.50	1	5.50	33.78	0.0021
AC	0.51	1	0.51	3.14	0.1366
BC	2.39	1	2.39	14.66	0.0123
A^2	33.07	1	33.07	203.17	< 0.0001
B^2	10.03	1	10.03	61.59	0.0005
C^2	24.25	1	24.25	148.98	< 0.0001
Residual	0.81	5	0.16		
Lack of fit	0.38	3	0.13	0.59	0.6781
Pure error	0.43	2	0.22		
Total	114.75	14			
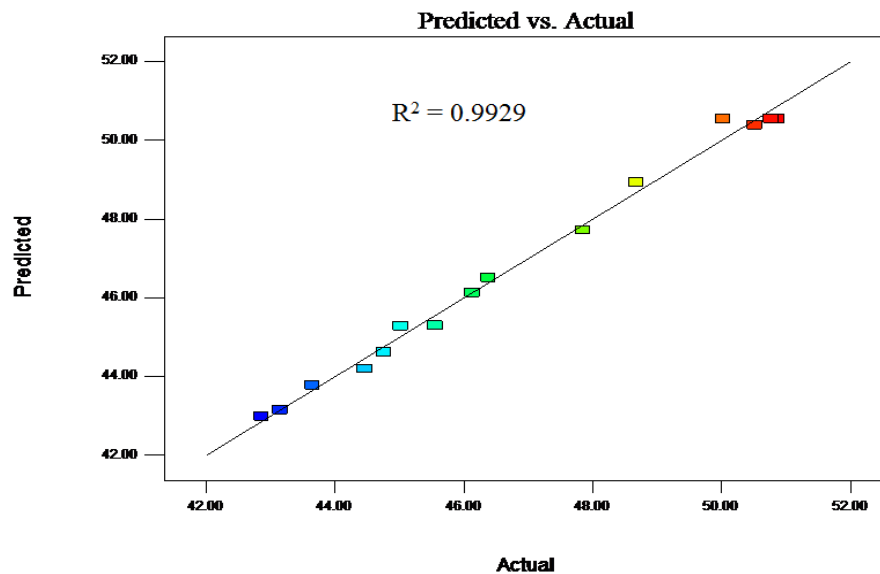
Figure 1: Relationships between predicted and actual values for complexing index.

Effects of main variables on complexing index

In this section, in order to more accurate investigate the effects of different factors, the 3-D plots of responses surface were used which were presented based on the related polynomial functions. The 3-D plots can illustrate the simultaneous effects of two independent factors on the response. **In Figure 2,** it can be seen the 3-D plots of simultaneous effects of lipid addition amount and stirring time on the variation of complexing index. Complexing index is increased by augmentation of lipid addition amount and stirring time. The increasing of complexing index is not severe by augmentation of lipid addition amount and stirring time from about 3.0 to 4.5% and 60 to75min, respectively. The possible explanation might be that the internal spiral structure of starch molecules was dominated by fatty acid molecules at a saturated degree when lipid concentration reached to a certain extent. As a consequence, the complex chances of starch and lipids decreased and the complexing index remained at a stable level. **In Figure 3,** it can be seen that the stirring time rising is caused to the continuous increasing of complexing index. However, the amount of complexing index is firstly increased and then decreased by bath temperature growing. Because the type-1 amorphous complex displays a melting temperature at about 94–100°C. The melting temperature of the amylose–fatty acid complex increases with the chain length of the fatty acid [16-18]. The amylose–lipid complex is melted during heating to the melting temperature, and the complex is reformed during cooling, but the complexing index is lower than before.

The contour plots shows the optimum conditions of responses R. Accordingly, it can be accessed to the certain ranges of responses by selection of desired amounts of effective factors. In fact, it can be done point prediction for each point of responses.
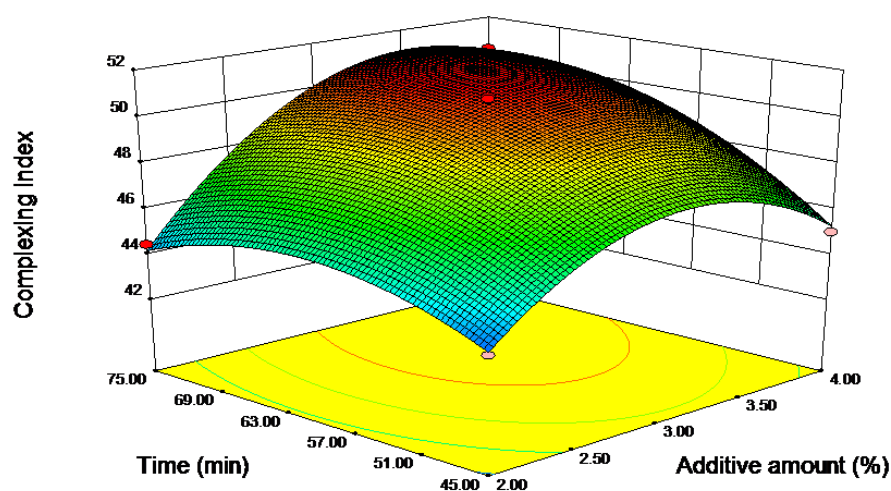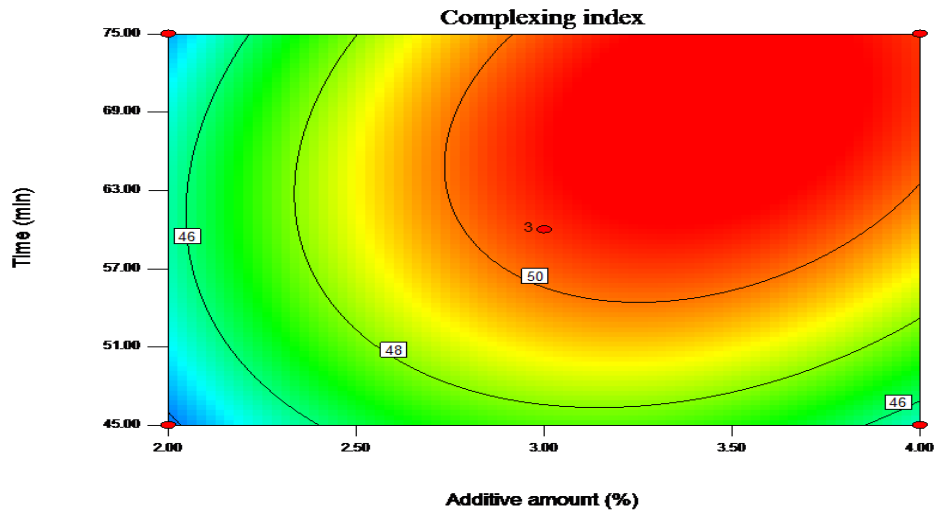
Figure 2: Response surface plots and contour plots showing the effects of lipid addition amount and stirring time on complexing index at water bath temperature 90 ℃.
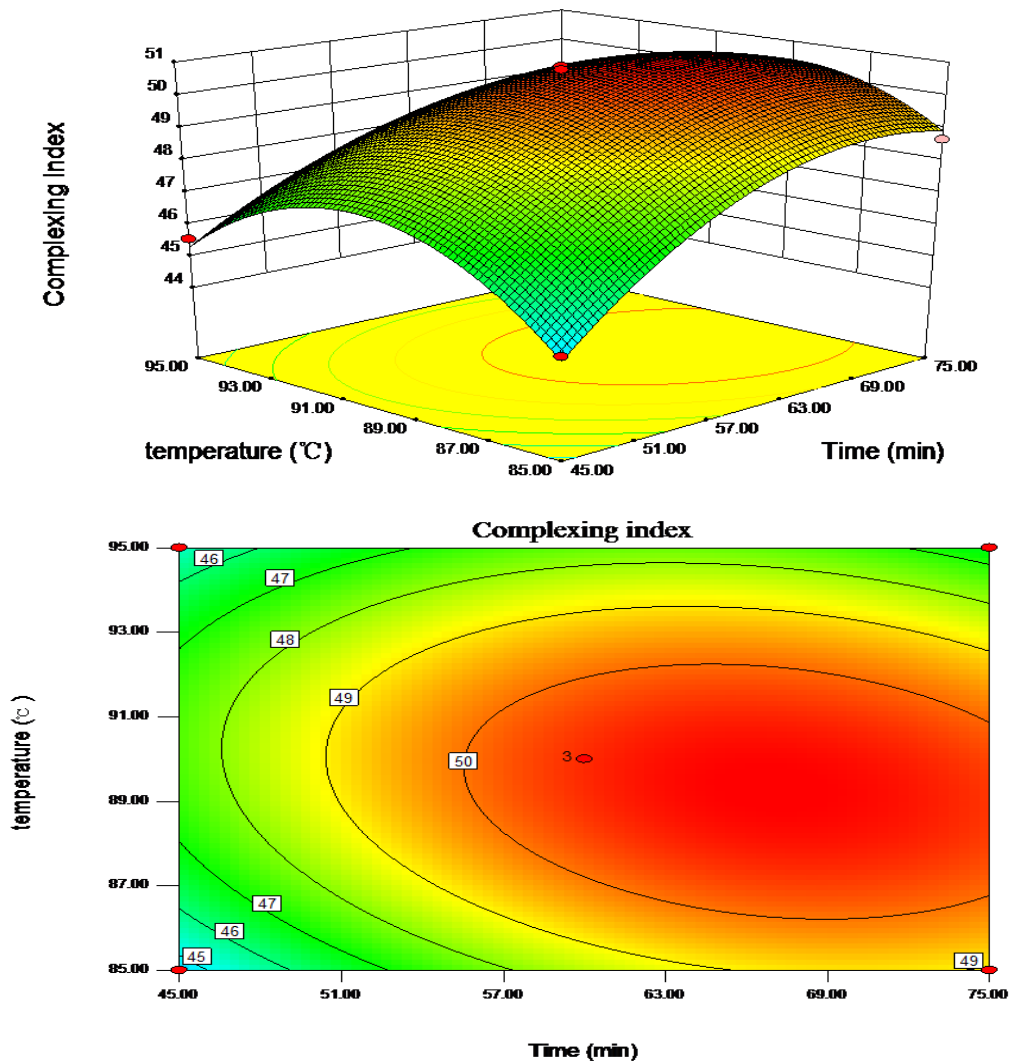

Figure 3: Response surface plots and contour plots showing the effects of stirring time and water bath temperature on complexing index at lipid addition amount of 3%.

Optimization of reaction conditions and model verification

The response surface methodology was employed to design and optimize the parameters of the complexation of starch and soybean lecithin. The lipid addition amount of 3.46 %, stirring time of 69.46 min, and water bath temperature of 88.94℃were found to be the optimum conditions to achieve the highest complexing index which reached 51.4763.

For the purpose of testing the reliability of the results and considering the practical convenience of the operation at the same time, the parameters were adjusted as follows: lipid addition amount of 3.5 %, stirring time of 70 min, and water bath temperature of 89℃. Three parallel experiments were taken under this condition and the results that the actual complexing index was 51.4429 had a small relative error compared to theoretical prediction which indicated that the complex process parameters obtained by response surface methodology were accurate, reliable and practical.

Conclusion

A regression model of the relations between complexing index and influencing factors (the amount of lipid, stirring time, and water bath temperature) was obtained by quadratic regression design. Three key influencing factors and their interaction were discussed based on response surface and contours of the regression model, and optimum technological conditions were as follows: the amount of lipid, 3.5 %, stirring time, 70 min, and water bath temperature, 89 ℃, the complex degree was 51.4429 obtained under this condition.

Considering the interaction between different operating conditions, it is important to establish a reasonable and effective model for the systematic analysis of the parameters using response surface methodology. It had been verified that the results under the optimal conditions obtained from the model fitted well with the regression predict value which could fully demonstrate the accuracy of the results obtained from the model, and meanwhile providing references for the research of starch-lipid complexation technology.

Acknowledgments

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