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Research Article

Preparation of Slowly Digestible Starch by Citric Acid-hydrolysis with Autoclaving

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Abstract: Aim of study is to produce Slowly Digestible Starch (SDS) by a combination of citric acid-hydrolysis and autoclaving from raw corn starch. The effects of citric acid concentration, acid hydrolysis time, temperature and refrigeration and retro gradation time on the formation of SDS were investigated. The optimal process conditions optimized by Box-Benhnken's central composite design and response surface analysis was as follows: concentration of citric acid is 0.08 M, acid hydrolyzed at 116°C for 14.5 min, and then stored at 0°C for 72 h. Under these optimal conditions, the content of SDS peaked at 19.38%.

Keywords: Autoclaving, citric acid-hydrolysis, slowly digestible starch

INTRODUCTION

Slowly Digestible Starch (SDS) can be digested at a decreased rate and absorbed in the human small intestine. According to *in vitro* conditions, SDS is defined as the amount that can be digested by α-amylase within 20-120 min based on starch bioavailability (Englyst *et al*., 1992). SDS has a variety of physiological functions:

- Compared to ordinary starch, SDS is less effective at simulating insulin secretion, which may help to regulate blood sugar levels in the body, maintain insulin function, improve insulin sensitivity and avoid hyperinsulinemia and insulin resistance metabolic syndrome.
- SDS is slowly digested and absorbed in the small intestine, slowly releasing energy and producing satiety for long time periods to help control obesity and maintain appropriate body weight.
- SDS can reduce blood triglycerides and cholesterol levels, reduce total fat deposition, reduce coronary heart disease and the incidence of micro vascular complications.
- SDS can improve bowel movement, promote fecal and intestinal toxin elimination and can reduce intestinal dysfunction and the incidence of colon cancer.
- SDS can be used as a carbohydrate source for athletes, in particular, marathon and other long-distance athletes, to maintain the runners' endurance (Liao *et al*., 2007; Shin *et al*., 2004, 2005; Lehmann and Robin, 2007; Guraya *et al*., 2001; Han *et al*., 2006).

Currently, SDS has not been used in commercial production, but the study of the preparation and the characteristics of its products have been previously reported. Han and Bemiller (2007) prepared SDS using raw corn starch, waxy corn starch and potato starch as raw material and by using chemical cross-linking and etherification methods. The percentage of SDS obtained from the starting samples was more than 30%, and the physical property of the SDS product was characterized; however, the SDS produced by this method was toxic and could not be used in the food industry. SDS was prepared by debranching enzymes by Guraya *et al*. (2001) and Shin *et al*. (2005). Additionally, SDS was prepared by autoclaving (Shin *et al*., 2003) and microwave heating (Anderson and Guraya, 2004). Han prepared SDS by enzymolysis and autoclaving (Han *et al*., 2006).

In summary, most researchers used waxy cornstarch as raw material and used enzymatic and heat treatment methods to study the preparation of SDS. In China, waxy corn starch is quite limited, with the source being dependent on imports, while raw corn starch is widely available. Therefore, for this study we used raw corn starch as raw material and citric acid-hydrolysis with autoclaving to obtain edible SDS starch products to establish a foundation for the industrial production of edible SDS.

MATERIALS AND METHODS

Raw materials and reagents: Citric Acid, Porcine pancreatic α-amylase was obtained from Fluca; Disodium hydrogen phosphate, Sodium dihydrogen phosphate, Glycerol, NaOH, KOH were purchased from Beijing north Fine Chemicals Co., Ltd.; 3,5-

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Table 1: The corresponding relation between encode and factors

Encode	Citric acid concentration (X1) (M)	Acid hydrolysis temperature (X2) (°C)	Acid hydrolysis time (X3) (min)
-1	0.06	110	10
0	0.08	115	15
1	0.10	120	20

Table 2: Box-Benhnken's central composite design

Run	X1	X2	X3
1	-1	-1	0
2	-1	1	0
3	1	-1	0
4	1	1	0
5	0	-1	-1
6	0	-1	1
7	0	1	-1
8	0	1	1
9	-1	0	-1
10	1	0	-1
11	-1	0	1
12	1	0	1
13	0	0	0
14	0	0	0
15	0	0	0

Dinitro salicylic acid was purchased from Shanghai Chemical Reagent Co., Ltd. Sinopharm.

Methods:

Preparation of SDS: A total of 30 g of starch was dissolved in 300 mL of different concentration (0.04, 0.06, 0.08, 0.1 and 0.12 M, respectively) of citric acid solution, magnetically stirred for 30 min and subsequently placed into an autoclaving pot and heated to the required temperature (105, 110, 115, 120 or 125°C, respectively) for different time (5, 10, 15, 20 or 25 min, respectively). After washing and centrifuging (6000 rpm, 5 min) 4 times, the precipitate was collected and placed at 0°C for refrigeration and retrogradation for different time (24, 72, 120, 168 or 216 h, respectively). The precipitate that passes the 100 mesh sieve was crushed and subsequently dried at 60° C for 20 h.

The effect of citric acid concentration on SDS content: Five concentrations (0.04, 0.06, 0.08, 0.1 or 0.12 M, respectively) were chosen while other conditions remained constant: the starch concentration was 10%, acid hydrolysis temperature was 120°C, the acid hydrolysis time was 20 min, refrigeration and retro gradation conditions were 0°C for 24 h, and the drying conditions were 60°C for 20 h, over a 100-mesh sieve after crushing.

The effect of acid hydrolysis time on the content of SDS: Five time intervals were chosen (5, 10, 15, 20 or 25 min, respectively). The citric acid concentration was 0.08 M while other conditions were kept as in section.

The effect of acid hydrolysis temperature on SDS content: Five conditions were chosen (105, 110, 115, 120 or 125°C, respectively). The citric acid concentration was 0.08 M; the acid hydrolysis time was 15 min, while other conditions were kept as in section below.

The effect of refrigeration and retrogradation time on SDS content: Five time intervals were chosen (24, 72, 120, 168 or 216 h, respectively). The citric acid concentration was 0.08 M, the acid hydrolysis time was 15 min, the acid hydrolysis temperature was 115°C, while other conditions were kept as in section below.

Box-Benhnken's central composite design: According to the results of optimization from the single-factor test and using the central composite design of Box-Benhnken, we further applied three factors and three levels of RSM (Response Surface Methodology) analysis to the test. The favorable choice on the formation of SDS using the three factors X1, X2, X3 through the central composite experiment was used to determine the best parameters. The corresponding relation between encode and factors is shown in Table 1, the Box-Benhnken's central composite design is shown in Table 2.

Determination of the content of SDS: The content of SDS was determined by an improved method of Guraya *et al*. (2001) and Lehmann and Robin (2007). Briefly, 100 mg of sample was dissolved in phosphate buffer (0.1 M, pH 6.9, 15 mL), boiled for 15 min in a water bath, cooled to 37°C, and mixed with 10 mL of porcine pancreas α-amylase (200 U/mL, 37°C) in a water shaker (150 rpm, 10 h) at 37°C. Maltose was produced by hydrolysis using the DNS method to measure and calculate, using a standard curve, the maltose concentration and to convert to the SDS concentration. The formula used is as follows:

$$SDS\% = (B - A)\,C \times 100\%$$

where,

A = The amount of maltose released after 1 h from enzymolysis of the sample
B = Total starch (maltose content)
C = The amount of maltose released after 10 h from enzymolysis of the sample

X-ray diffraction to determine crystallinity: We determined the crystallinity of the SDS powder samples and the raw corn starch by X-ray diffraction curve analysis. The test conditions included the following: the tube voltage was 40 kV, the current was 40 mA and the diffraction angle was 2^{θ} = 10-80°, the scanning rate was 5°/min, and the step was 0.01°. The method for calculation of crystallinity was performed according to Zhang's method (Zhang *et al*., 2001).

RESULT ANALYSIS

The effect of citric acid concentration on the content of SDS: It was reported that Zobel (1988) amylose molecules aggregated through a combination of hydrogen bonding into the crystalline structure. The α-1, 4 bonds is difficult to hydrolyze by acid because of the difficulty in acid accessibility. The α-D (1, 4) bond and α-D (1, 6) bond of the branched-chain starch molecules in the amorphous region are more easily accessible by acid and hydrolyzed. When starch is hydrolyzed by citric acid, initially a rapidly hydrolyzed amylopectin-containing amorphous zone is formed, followed by slow hydrolysis of amylose in the crystalline part.

From Fig. 1, we show that at the same acid hydrolysis time and temperature conditions, an increase in the citric acid concentration to 0.08 M led to an increase in the contribution that the freezing step played in the retrogradation of amylose, leading to increased accumulation of SDS to 17.85%.

The effect of acid hydrolysis time on the content SDS: As shown in Fig. 2, acid hydrolysis time had a greater impact on the formation of SDS than did the acid concentration.

When the acid hydrolysis time was kept at 10 and 15 min, the contents of SDS reached 18.18 and 18.36%, respectively. The reason for this is that too long or too short of an acid hydrolysis time may not be conducive for the starch molecules, after gelatinization, retrogradation and crystallization, to form SDS. Too short of an acid hydrolysis time yields gelatinization of more long-chain starch molecules. When the orientation of starch molecules retrogradation is slow, crystallization of starch molecules does not occur. Too long of an acid hydrolysis time will yield gelatinization of short-chain starch molecules. So it difficult for gelatinized starch molecules to form crystalline SDS.

When the native starch hydrolysis time is kept between 10 to 15 min, amylose accumulation plays an important role on starch retrogradation and occurs at relatively high frequency. The resulting chain length that is formed is appropriate, and it is therefore easy to gelatinized starch molecules during retrogradation, leading to better crystallization and increased SDS content.

The effect of acid hydrolysis temperature on the content of SDS: The acid hydrolysis temperature is also an important factor affecting the formation of SDS.

Figure 3 shows that as the temperature increases, the degree of starch hydrolysis increases and therefore the number of amylose molecules increases, making it easier for starch to undergo retrogradation and crystallization. When the acid hydrolysis temperature is 115°C, the content of SDS increases up to 18.22%. As the temperature continues to raise, the content of SDS decreases slightly. This is because after the temperature is raised further, the citric acid can lead to amylose hydrolysis reducing the number of available starch molecules.

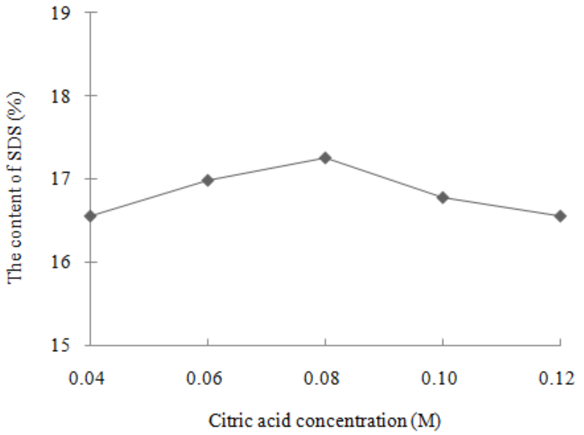


Fig. 1: Effect of citric acid concentration on the content of SDS

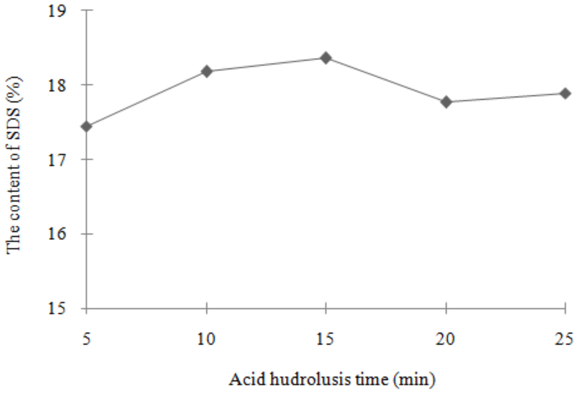


Fig. 2: Effect of acid hydrolysis time on the content of SDS

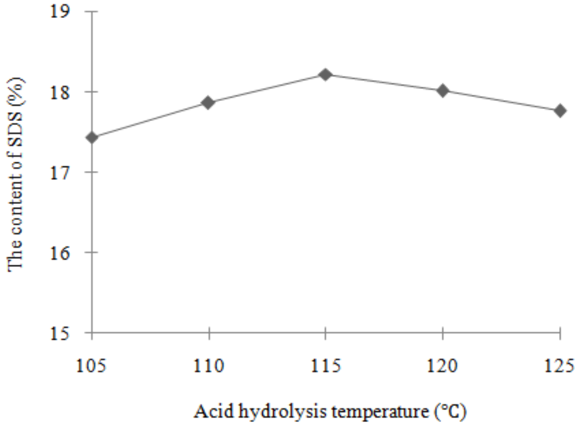


Fig. 3: Effect of acid hydrolysis temperature on the content of SDS

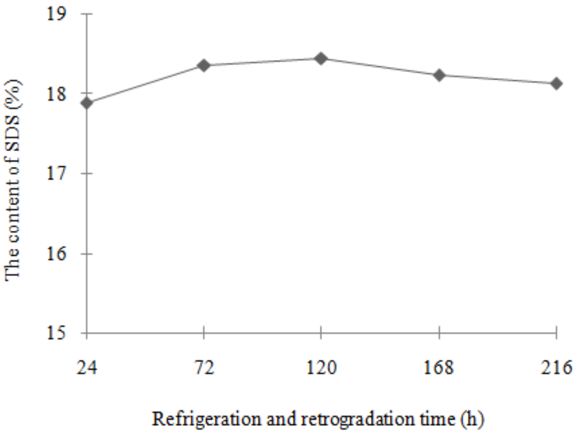


Fig. 4: Effect of refrigeration and retrogradation time on the content of SDS

The effect of refrigeration and retrogradation time on the content of SDS: SDS is formed mainly by re-crystallization of the gelatinized starch molecules. The process of crystallization requires low temperature, and the time of refrigeration and retrogradation time is essential.

As shown in Fig. 4, with prolonged refrigeration and retrogradation time, the content of SDS increases. When the refrigeration and retrogradation time was 120 h, the content of SDS was the highest. However, when the refrigeration and retrogradation time was longer than 72 h, the increase in content of SDS was not apparent. The reason for this may be that after acid hydrolysis of the starch molecules, retrogradation may have occurred primarily in the previous 72 h, with the prolonged time allowing for resuscitation and retrogradation of starch molecules in the crystallized form to stabilize.

Box-Benhnken central composite design results and the response surface analysis: By using single-factor test results, we selected the optimal factors from the citric acid concentration, acid hydrolysis temperature, acid hydrolysis time and refrigeration and retrogradation time that significantly increased the SDS concentration. To obtain the best combination of factors and their levels, we used a Box-Benhnken central composite design and further used three factors and three levels of the RSM (Response Surface Methodology) to analyze the experiments. We investigated the effect that citric acid concentration, acid hydrolysis temperature and acid hydrolysis time had on the quality of the SDS. Fifteen test points could be divided into two categories, one being the factorial point. There is 12 factorial points because the variable values in the X1, X2, X3 posed by the three-dimensional vertices. The focal point of the region was zero. To estimate the test error, zero experiments were repeated three times. We used SAS software for data processing. We investigated the effect of the larger main effect, the main factors and interaction effects to obtain the optimal process parameters (Table 3).

Table 3: Box-Benhnken central composite design and the content of SDS

Run	X1	X2	X3	Y
1	-1	-1	0	17.76
2	-1	1	0	17.82
3	1	-1	0	17.71
4	1	1	0	18.47
5	0	-1	-1	17.28
6	0	-1	1	16.83
7	0	1	-1	18.07
8	0	1	1	17.27
9	-1	0	-1	17.85
10	1	0	-1	16.96
11	-1	0	1	17.27
12	1	0	1	16.65
13	0	0	0	19.27
14	0	0	0	19.22
15	0	0	0	19.21

According to the SDS concentration, use the SAS software obtained regression equation (coded) are as follows: $Y1 = 19.32667 - 0.11375\times X1 + 0.25625\times X2 - 0.2675\times X3 - 0.783333\times X1^2 + 0.175\times X1\times X2 + 0.0675\times X1\times X3 - 0.603333\times X2^2 - 0.0875\times X2\times X3 - 1.360833\times X3^2$

Use of the SAS program for analysis of variance shown in Table 4.

The results of the analysis of variance regression equation show that the established model is significant ($p<0.5$) with $X1^2$, $X3^2$ having an impact that was extremely significant ($p<0.01$). The effects X1, X2, X3, X1×X2, $X2^2$ on SDS concentration were also significant ($p<0.5$). The effect of X1×X3, X2×X3 on the SDS concentration had no significant effect ($p>0.5$). Therefore, the impact that each specific test factor has on the response is not a simple linear relationship, but rather a quadratic paraboloid. Teliability analysis of the model is shown in Table 5.

The square of multiple correlation coefficient $R^2 = 0.8439$ in the table shows that the model established by these three factors and quadratic terms could explain 84.39% of the changes in the SDS content. Therefore, the regression equation provides a more appropriate model for citric acid-hydrolysis with autoclaving to prepare SDS.

Figure 5 is the response surface and contour line of the SDS response surface analysis. From the figure, one can see that the effect changes in trends respond to various factors, and there is a maximum in the regression model.

On the regression equation, one can take a first-order partial derivative and organize it as follows:

$$-0.11375 - 1.566666X1 + 0.175\ X2 + 0.0675X3 = 0$$

$$0.25625 + 0.175X1 - 1.206666\ X2 - 0.0875X3 = 0$$

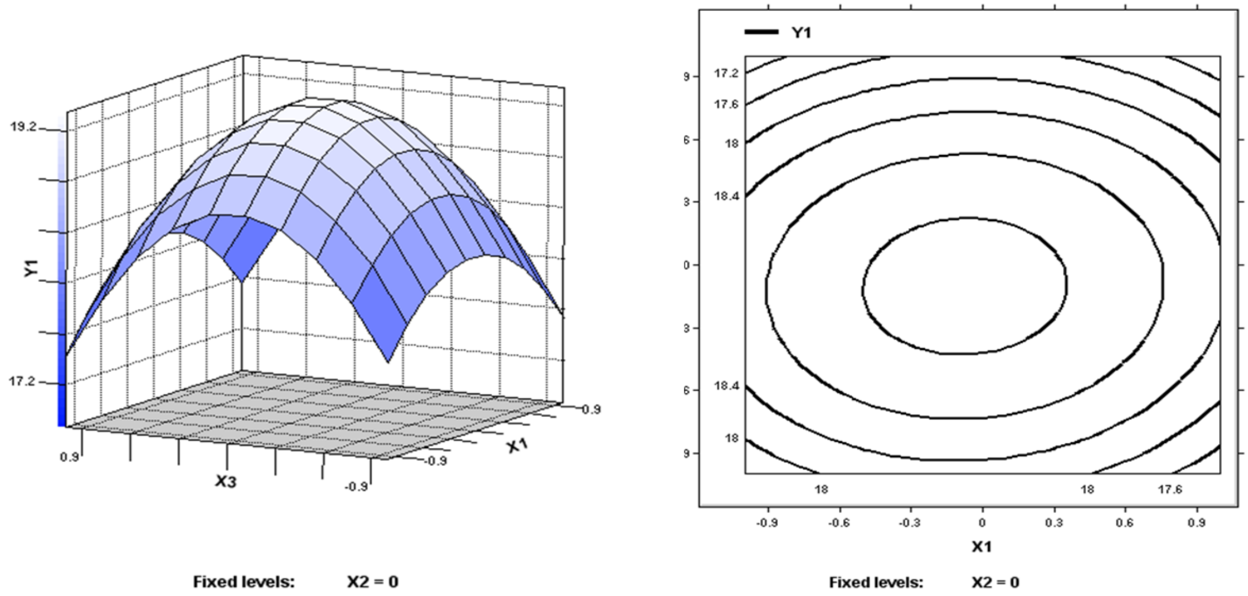
$$-0.2675 + 0.0675X1 - 0.0875\ X2 - 2.721666X3 = 0$$

The solution was: $X1 = -0.047$, $X2 = 0.208$, $X3 = -0.107$. The code value of the stable point (X1,

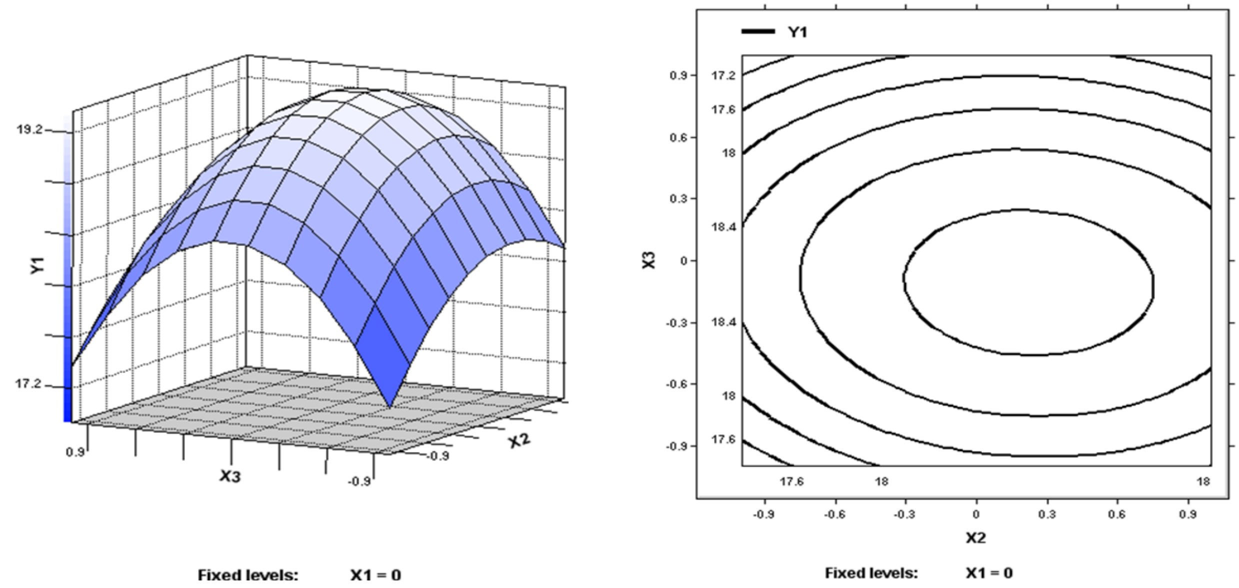
Table 4: Variance analysis result of response surface test

	Master model					Predictive Model				
Source	D.F.	S.S.	M.S.	F	Pr>F	D.F.	S.S.	M.S.	F	Pr>F
X1	1	0.103513	0.103513	0.820757	0.406497	1	0.103513	0.103513	0.820757	0.406497
X2	1	0.525312	0.525312	4.165235	0.096759	1	0.525312	0.525312	4.165235	0.096759
X3	1	0.572450	0.572450	4.538991	0.086350	1	0.572450	0.572450	4.538991	0.086350
X1×X1	1	2.265641	2.265641	17.964410	0.008182	1	2.265641	2.265641	17.964410	0.008182
X1×X2	1	0.122500	0.122500	0.971310	0.369612	1	0.122500	0.122500	0.971310	0.369612
X1×X3	1	0.018225	0.018225	0.144507	0.719453	1	0.018225	0.018225	0.144507	0.719453
X2×X2	1	1.344041	1.344041	10.656980	0.022334	1	1.344041	1.344041	10.656980	0.022334
X2×X3	1	0.030625	0.030625	0.242828	0.643047	1	0.030625	0.030625	0.242828	0.643047
X3×X3	1	6.837664	6.837664	54.216260	0.000726	1	6.837664	6.837664	54.216260	0.000726
Model	9	10.677580	1.186398	9.407022	0.011872	9	10.677580	1.186398	9.407022	0.011872
Error	5	0.630592	0.126118			5	0.630592	0.126118		
Total	14	11.308170				14	11.308170			

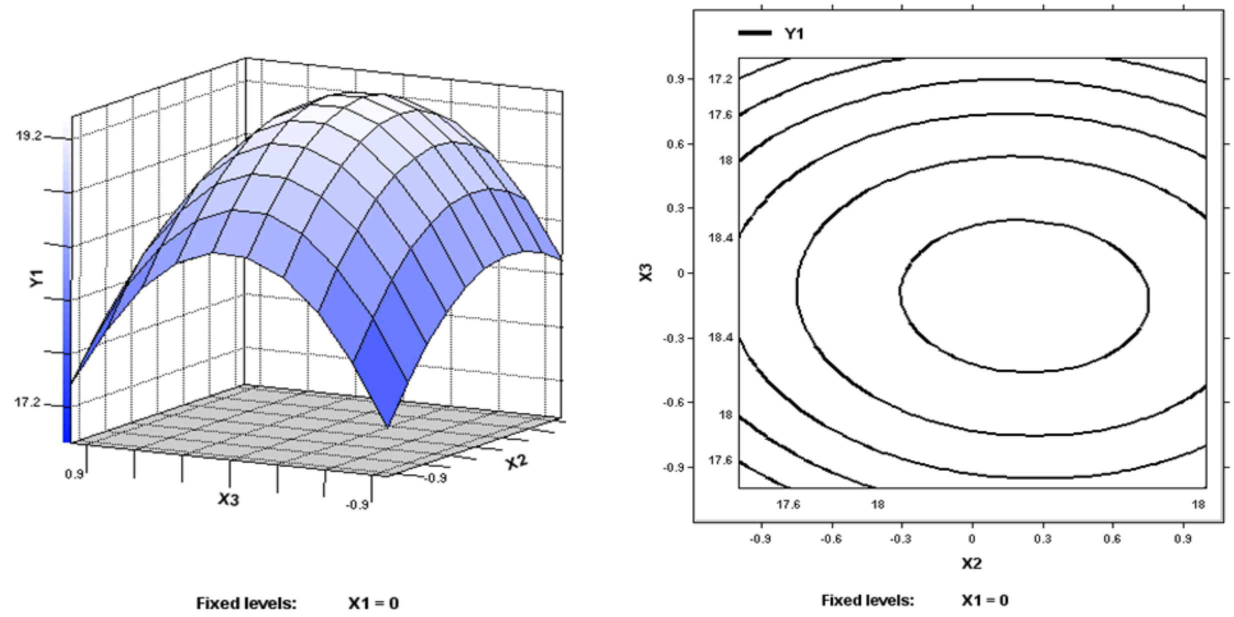
D.F.: Degree of freedom; S.S.: Sum of square; M.S.: Mean square



(a) Y1 = f (X1, X2)



(b) Y1 = f (X1, X3)



(c) Y1 = f (X2, X3)

Fig. 5: Figure of response surface and contour line on SDS response surface analysis

Table 5: Reliability analysis of the model

	Master model	Predictive model
Mean	17.861330	17.861330
R-square	94.420000%	94.420000%
Adj. R-square	84.390000%	84.390000%
RMSE	0.355131	0.355131
CV	1.988269	1.988269

X2, X3) was (-0.047, 0.208, -0.107). From this, we determined the citric acid concentration (X1) = 0.079 M, acid hydrolysis temperature (X2) = 116.04°C and acid hydrolysis temperature (X3) = 14.465 min, yielding a theoretical maximal estimated SDS content of Y1 = 19.37%. Taking into account the actual operation of the facilities, we determined a citric acid concentration of 0.08 M, acid hydrolysis temperature of 116°C, and acid hydrolysis time of 14.5 min.

To validate the projected results, we used the optimal parameters determined for the center test conditions of central composite design and repeated the test five times. The SDS-center trial received a level of 19.22±0.12%. Since the three-center trial error result of central composite design was relatively small, the method illustrates that the results are valid. The optimal parameters yielded an SDS content of 19.33±0.15%, which is close to the predicted value of Y1 = 19.37%. Thus, the description of the regression equation for the preparation of SDS provides a more appropriate model.

DISCUSSION

To date, researchers have used primarily waxy corn starch as the raw material for enzymolysis (mainly isoamylase, pullulanase and α-amylase) to change the chain length or molecular weight of starch and have used re-crystallization and retrogradation to prepare SDS. As in SDS that has been prepared by a debranching enzyme as measured by Guraya *et al*. (2001) and Lehmann and Robin (2007). Nian Shin etc., Guraya *et al*. (2001) used enzymolysis combined with autoclaving to prepare SDS. As the use of enzymes is expensive, the production cost is too high to be commercially feasible. Several groups have used chemical modification to prepare SDS, such as 2006 Han and Bemiller (2007) and Sang *et al*. (2007) who examined the use of common corn starch, waxy corn starch, potato starch, etc. and used chemical cross-linking and esterification preparation methods to prepare SDS. Laboratory samples with SDS concentrations of more than 30% have been obtained and the physical characteristics of these SDS samples have been studied. However, the toxic chemicals used during the preparation, (i.e., phosphorus oxychloride, propylene oxide, etc.) makes the SDS toxic and unsuitable to be used in the food processing industry.

Using rice starch as raw material and the citric acid pressure heat treatment, the highest SDS content achieved has been only 14.1%, as reported by Sang *et al*. (2007); the study used a wide range of sources of common corn starch as raw material. Additionally, the study also used the more economical food citric acid and used a pressurized hot acid solution to study the preparation SDS. Through a pressurized hot acid solution, the SDS content was 19.38%.

The use of a single-factor test to optimize the citric acid concentration, acid hydrolysis temperature, acid hydrolysis time and refrigeration and retro gradation time. The results show that the citric acid concentration, acid hydrolysis temperature and acid hydrolysis time had a great impact on the formation of SDS. The citric acid concentration had the greatest impact, while the frozen retrogradation time factor did not significantly impact SDS formation.

According to the single-factor test results, we selected the level of citric acid concentration, acid hydrolysis temperature and acid hydrolysis time. Using the Box-Benhnken central composite test, we use three factors and three levels of the RSM (Response Surface Methodology) analysis of experiments. The results show that the established model was statistically significant ($p<0.5$). A verification experiment with the best parameters was prepared using the SDS results, and the predicted values were found to fit well. Thus, the regression equation using the citric acid solution prepared by hot pressing SDS provides a more appropriate model.

CONCLUSION

After determining a citric acid concentration, the time and temperature of acid hydrolysis, and the refrigeration and retrogradation time single-factors were used in the center of the Box-Benhnken combination of tests. The results show that the model was statistically significant ($p<0.5$). Validation experiments showed that the best parameters are a citric acid concentration of 0.08 M, an acid hydrolysis temperature of 116°C, and an acid hydrolysis time of 14.5 min. The prepared SDS content was 19.38% and the predicted values fit good. Therefore, the description regression equation for the citric acid solution prepared by hot pressure SDS provides a appropriate analysis model. The X-ray diffraction results showed that the degree of crystallinity decreased by 6.37% compared to the original SDS of corn starch.

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