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Optimization of the Slowly Digestible Starch Formation from Edible Canna Starch Modification with Beta Cyclodextrin

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Abstract

Slowly digestible starch is the starch fraction that is digested at a slow rate in the body, meaning it is broken down by the digestive enzyme in human body during 20 to 120 min. after eating. Recently, slowly digestible starch (SDS) and resistant starch (RS) are widely studied worldwide because of their various positive health effects: releasing glucose at a slow rate, thereby maintaining sufficient blood glucose, glycemic index and insulin levels, reducing the risk of Type II diabetes, etc... The goal of the research was to identify the optimal conditions for maximizing the production of SDS from edible canna starch by using β-cyclodextrin with four factors examined: water content, β-cyclodextrin content, reaction temperature, and reaction time. When amylose in starch interacts with β-cyclodextrin through their hydrophilic shells, amylose-β-cyclodextrin (amylose-β-CD) and amylose-β-CD-lipid complexes are formed. These complexes exhibit a V-type crystalline structure characterized by low stability. It facilitates an increase in the production of SDS. The results showed that with a water content of 81.3%, β-cyclodextrin content of 3.1%, reaction temperature of 36 °C, and reaction time of 1.8 hours, the SDS content was obtained from edible canna starch up to 44.88%.

Keywords: Edible canna starch, slowly digestible starch, optimization, β-cyclodextrin, SDS.

1. Introduction

Starch is a complex carbohydrate found in many foods, particularly in cereals such as wheat, rice, and corn, as well as in potatoes and other starchy roots. Starch occurs widely in nature and is the second largest biomass on earth after cellulose and one of the most abundant bio-renewable materials. The properties of native starch do not always meet the requirements for a multitude of industrial applications [1]. Based on the rate and extent of digestion, starch can be classified into 3 types: rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS), which were first introduced by H. Englyst *et al*. (1992) [2] to reflect the rate of starch digestion in vivo. The starch fraction digested within 20 min of incubation is classified as RDS; the starch fraction digested within 20 - 120 min corresponds to SDS; and the remaining fraction, which is not digested further, is RS. This value may be an underestimation, as some starches were considered to take closer to 4 h to pass out of the small intestine [3-4].

SDS refers to a starch fraction with a slow digestion rate in the small intestine. SDS has the potential to ensure stable postprandial glucose metabolism, lower the risk of diabetes, and also ensure superior mental and physical performance in terms of health effects on the human body [5]. Foods containing SDS could cause an improvement in the carbohydrate metabolism and facilitate a concomitant reduction in the insulin requirements of insulin-treated type 2 diabetes mellitus (T2DM) patients [6-7].

There were several ways to form SDS from starch. Physical modifications include hydrothermal (heat-moisture and annealing), microwave, ultrahigh pressure (UHP), irradiation, and ultrasonic treatment [8]. Chemical modifications are mostly practiced for food starches, generally by derivatization such as etherification, esterification, cross-linking, oxidization, and acid hydrolysis of starch. Enzymatic modification mainly involves treatment of starch using hydrolyzing enzymes [9]. Among those, physical methods were considered more natural and were safe. The physical modification methods used to produce SDS included hydrothermal, autoclaving, microwaving, and polymer entrapment methods as well as using β-cyclodextrin.

β-cyclodextrin (β-CD) is a cyclic and non-reducing functional oligosaccharide that consists of D-glucose units with a-1,4 glycosidic bonds in a doughnut-shaped ring. [10] Its aperture, with its hydrophobic core, can form inclusion complexes with small organic and inorganic molecules in aqueous

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solutions, while its outer hydrophilic shell can interact with the hydroxyl groups of carbohydrates, such as starch molecules. [11] When amylose in starch interacts with β-cyclodextrin through their hydrophilic shells, amylose-β-cyclodextrin (amylose-β-CD) and amylose-β-CD-lipid complexes are formed. These complexes exhibit a V-type crystalline structure characterized by low stability. It facilitates an increase in the production of SDS, while simultaneously reducing the proportion of RS with a type B crystalline structure [12]. Furthermore, the V-type crystalline structure is associated with a melting temperature of over 100 °C. This higher melting temperature indicates that β-CD is suitable as a healthier denaturant instead of lipids for the preparation of SDS with high thermal stability [12].

In Vietnam, edible canna (*Canna Edulis Kerr*) was introduced by the French in the 19th century. Nowadays, edible canna is grown in many regions across the country, including Son La, Dien Bien, Yen Bai, Tuyen Quang, Lao Cai, Hoa Binh, Thai Binh, Cao Bang, Thanh Hoa. There is little application of edible canna starch and the main product of edible canna is edible canna noodle. Edible canna starch is characterized by its large granule size (average size of 50-60 μm), high gelatinization temperature (73-74 °C), which depends significantly on the granule size and distribution of the starch. Edible canna starch has a lower swelling power compared to many starches from other tubers like sweet potatoes, manioc, and potatoes, but its gelatinized starch has a higher clarity [13-14].

In the previous study, the effects of β-cyclodextrin on the formation of SDS from edible canna starch were studied. The purpose of this research was to find the optimum condition for SDS formation from edible canna starch.

2. Materials and Methods

2.1. Materials

Edible canna starches were obtained from Vietnam - Korea institute of science and technology (VKIST). D-Glucose Assay Kit (GOPOD Format) was purchased from Megazyme. β-Cyclodextrin was purchased from Shanghai Zhanyun Chemical Co. Ltd. Pullulanase M2 (*Bacillus licheniformis*) 1000 U/mL, Amyloglucosidase (*Aspergillus niger*) was purchased from Megazyme. α-Amylase and from porcine pancreas were purchased from Sigma-Aldrich

2.2. Modification of Edible Canna Starch with β-Cyclodextrin

Starch, β-cyclodextrin with the content ranged from 2% to 4% (w/w) and water with the content ranged from 60% - 90% (w/w) were mixed gently and then were fully gelatinized. The resulting mixtures were gelatinized in conical flasks using a high-pressure steam sterilization pot at 100 °C in water vapor for 30 minutes. The samples were then hermetically sealed and subjected to moisture equilibration at temperatures ranging from 18 °C to 60 °C for durations of 1 to 2.5 hours to prepare starches physically modified by β-cyclodextrins (β-CDs). The resultant samples were then dried in an air oven at 55 °C and milled to pass through a 100-mesh sieve for analysis. The modifying temperature, beta-cyclodextrin content, water content and time were studied based on optimization design.

2.3. Digestibility of Starch

The digestibility was determined according to the procedure of H. Englyst *et al*. (1992) [2] and Ming Miao *et al.* (2014) [15] with a slight modification.

To prepare Enzyme Solution I, the amyloglucosidase solution (0.035 ml) was diluted to 1.5 ml with distilled water. Enzyme Solution II was prepared by suspending porcine pancreatic α-amylase (12.0 g) in water (80.0 ml) with magnetic stirring for 10 min, and then centrifuging the mixture for 10 min at 1500 g. Finally, a portion (13.5 ml) of the supernatant was transferred to a beaker. Enzyme Solution III was prepared immediately before using by mixing water (1.0 ml), Enzyme Solution I (1.5 ml) and Enzyme Solution II (13.5 ml).

The starch sample (100 mg) was dissolved in 7.5 ml of phosphate buffer (0.2 M, pH 5.2) by vortex mixing. After the starch solution was equilibrated at 37 ºC for 5 min, 10 glass balls (3 mm diameter) and Enzyme Solution III (2.5 ml) were added. The samples were then shaken in a 37 ºC water bath at 120 rpm. Aliquots of hydrolyzed solution (0.5 ml) were taken at different time intervals and mixed with 4.5 ml of absolute ethanol to deactivate the enzymes. The glucose content of the hydrolysate was determined using the glucose oxidase/peroxidase assay kits. Each sample was analyzed in triplicate. SDS calculated as follows:

$$\%SDS = (G120 - G20) \times 0.9 \times 100 \quad (1)$$

where

SDS is slowly digestible starch (%);

G20 is Total glucose after 20 min. of digestion;

G120 is Total glucose after 120 min. of digestion.

2.4. Total Glucose Determination

The glucose content of the hydrolysate was determined using the glucose oxidase/peroxidase assay kits (GOPOD). The color intensity of the mixture was directly proportional to the concentration of reducing sugar. The absorbance was measured at a wavelength of 510 nm.

2.5. Optimization and Modalization Design

The statistical software Design-Expert 11 was used to perform experimental design and optimization base on Box-Behnken design

2.6. Statistical Analysis

All experimental results data were analyzed using Microsoft Excel. Statistical analysis was carried out for all the sourced data using IBM SPSS Statistics 27 was used to perform analysis of variance (ANOVA). The Duncan's multiple range test was applied to determine the differences between the means, testing on the significance level of p less than 0.05. All experiments were repeated three times.

3. Results and Discussion

From the previous survey of influencing factors, there were 4 factors affecting the formation of SDS from edible canna starch such as water content, β-cyclodextrin content, temperature, time, shown in Table 1.

- Water content: 60% (w/w) - 90% (w/w)
- β - CD content: 2% (w/w) - 4% (w/w)
- Temp.: 18 ^oC - 60 ^oC
- Time: 1h - 2.5h

Table 1. Level of factors

Level	Factor			
	X1 (%)	X2 (%)	X3 (^oC)	X4 (hour)
Basic (0)	75	3	39	1.75
Upper level (+)	90	4	60	2.5
Lower Level (-)	60	2	18	1

To evaluate the influence of various factors on the starch modification process using β-cyclodextrin, a second-order orthogonal experimental design was applied with four encoded factors:

- X1 - Water content (%)
- X2 - β-cyclodextrin content (%)
- X3 - Temperature (^oC)
- X4 - Time (h)

According to the Box-Behnken model with 4 influencing factors, the number of experiments was 27, including 3 center point experiments (Table 2). The second-order regression equation for the objectives is constructed as follows

$$y = b_0 + \sum_{j=1}^{k} b_j x_j + \sum_{j,u=1}^{k} b_{ju} x_j x_u + \ldots + \sum_{1}^{k} b_{jj} x_j^2 \quad (2)$$

where, y was the percentatge of SDS (%)

b_0 is the intercept

b_j was the linear coefficients

b_{ju} was the interaction coefficients

b_{jj} was the quadratic coefficients

X_1,X_2,X_3,X_4 were the encoded factors

Table 2. Experimental Matrix for Optimizing the Starch Modification Process of Edible Canna for SDS Production Using β-Cyclodextrin

No	X_1	X_2	X_3	X_4	Y
1	-1	-1	0	0	36.63
2	1	-1	0	0	43.74
3	-1	1	0	0	37.53
4	1	1	0	0	43.02
5	0	0	-1	-1	40.59
6	0	0	1	-1	41.22
7	0	0	-1	1	41.31
8	0	0	1	1	41.58
9	-1	0	0	-1	36.45
10	1	0	0	-1	44.64
11	-1	0	0	1	39.51
12	1	0	0	1	41.67
13	0	-1	-1	0	38.97
14	0	1	-1	0	39.33
15	0	-1	1	0	40.5
16	0	1	1	0	42.03
17	-1	0	-1	0	37.8
18	1	0	-1	0	43.02
19	-1	0	1	0	39.33
20	1	0	1	0	42.3
21	0	-1	0	-1	40.14
22	0	1	0	-1	40.14
23	0	-1	0	1	41.13
24	0	1	0	1	42.66
25	0	0	0	0	43.92
26	0	0	0	0	44.01
27	0	0	0	0	44.37

The encoded regression equation for forming SDS starch from edible canna starch using β-Cyclodextrin was as follows:

$$Y = 44.10 + 2.60A + 0.30B + 0.495C + 0.39D - 1.51AD - 1.98A^2 - 1.96B^2 - 1.69C^2 - 1.30D^2 \quad (3)$$

where A, B, C, D were encoded factors according to X_1, X_2, X_3, X_4 respectively.

The actual regression equation was

$$SDS = -69.234 + 1.728X_1 + 12.045X_2 + 0.322X_3 + 18.69X_4 - 0.134X_1X_4 - 0.009X_1^2 - 1.958X_2^2 - 0.004X_3^2 - 2.32X_4^2 \quad (4)$$

The determination coefficient (R^2) calculated was 0.9282. This indicated that model explains 92.82% of the variation in SDS depending on 4 influencing factors. Regarding the regression equation for slow-digesting starch (SDS) content, it was shown that the coefficients were ranked as $A > C > D > B$ (2.6 > 0.495 > 0.39 > 0.3). This suggested that water content had the greatest effect on the ability to produce slow-digesting SDS starch, followed by the reaction temperature with the second highest impact. The remaining two factors, β-cyclodextrin content and reaction time, had a lesser impact on the ability to form slow-digesting SDS starch (Fig. 1).

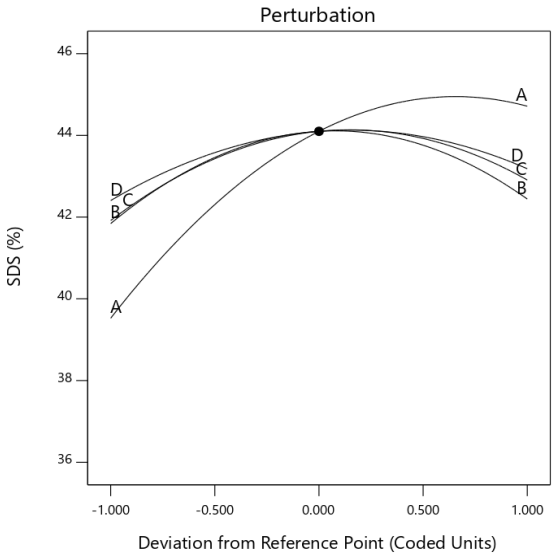

Fig. 1. Effects of 4 factors on the formation of SDS from edible canna starch using β -cyclodextrin.

Note: A, B, C, D were encoded factors according to water content (%), β-cyclodextrin content (%), temperature (°C), time (h) respectively

The relationship of 2 independent variables (water content and β -cyclodextrin content) and the formation of SDS from edible canna starch was showed in Fig. 2. The color gradients showed the content of SDS and the SDS content showed a gradual transition, represented by the color change from blue (lower values), through green and yellow to red (higher values). It was clearly seen that there was a nonlinear relationship between variables (water content and β -cyclodextrin content). The increase in water content could enhance the swelling and gelatinization of starch, increasing the likelihood of contact between starch molecules and β-cyclodextrin. Tian (2009) reported that the amylose-β-CD non-inclusion complex only formed during the cooling process of gelatinized starches [11]. Thus, it was inferred that the migration rate of starch and β-CD molecules was determined by the equilibrium between water content and starch/β-CD concentration. It was obviously shown that the SDS content increased with the increase in water content (A) and β-cyclodextrin content (B), then decreased. The interaction between water and β-cyclodextrin significantly impacted the amount of SDS formed, proving that both factors affected the digestibility of starch.

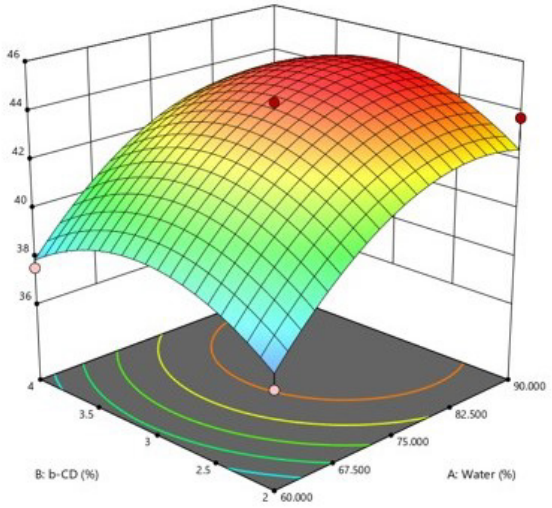

Fig. 2. Effects of water content and β -cyclodextrin content on the formation of SDS from edible canna starch

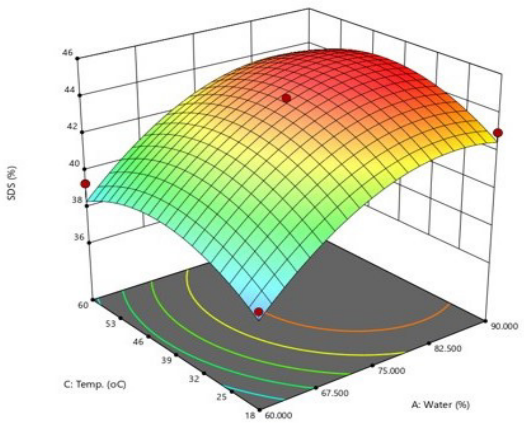

Fig 3. Effects of water content and temp. on the formation of SDS from edible canna starch

Fig. 3 represented the relationship of 2 independent variables (water content and temp.) and the formation of SDS from edible canna starch. Increasing water content and decreasing temperature increase the SDS content. This increase could be explained by the interaction of β-CD with amylose or starch molecules through the formation of a starch-β-CD non-inclusion complex [8]. Increasing water content promotes the swelling and gelatinization of starch. Meanwhile, decreasing temperature increases the chance of contact between starch β-CD and the formation of amylose-β-CD complex. It was indicated from Fig. 3 that the SDS content increased with the increase in water content *(A)* and decreased with an increase in temp. and the interaction between water content and temp. significantly affected the SDS formation.

Similarly, regarding the influence of temperature, 2 independent variables (time and temp.) and the formation of SDS from edible canna starch were presented in Fig. 4. Lower temperatures could promote nucleus formation and increase the yield of resistant starch (RS) [19]. In contrast, higher temperatures may not provide sufficient driving force for the interaction between starch and β-cyclodextrin molecules [11].

The Model *F*-value of 24.42 implies the model was significant. There was only a 0.01% chance that an *F*-value this large could occur due to noise (Table 3).

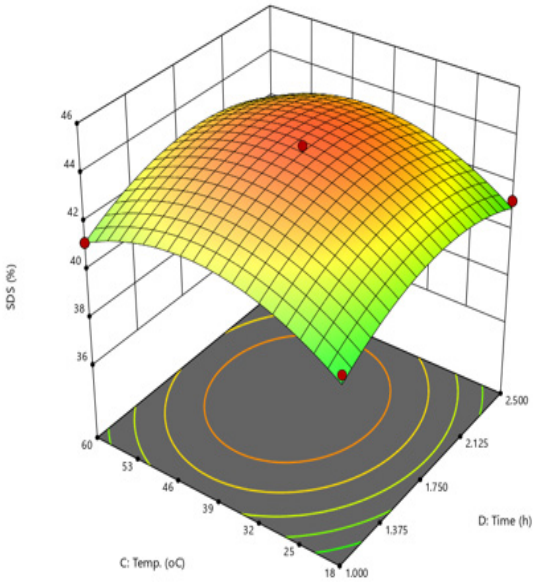

Fig. 4. Effects of time and temp. on the formation of SDS from edible canna starch using β -cyclodextrin

p-values less than 0.0500 indicate model terms were significant. In this case *A, C, AD, A², B², C², D²* were significant model terms. Values greater than 0.1000 indicate the model terms were not significant. If there were many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model.

Table 3. ANOVA for Reduced Quadratic mode

Response: SDS

Source	Sum of Squares	df	Mean Square	*F*-value	*p*-value	
Model	130.13	9	14.46	24.42	< 0.0001	significant
A-Water content	80.81	1	80.81	136.47	< 0.0001	
B-β-CD content	1.08	1	1.08	1.82	0.1946	
C-Temp.	2.94	1	2.94	4.97	0.0396	
D-Time	1.83	1	1.83	3.08	0.0971	
AD	9.09	1	9.09	15.35	0.0011	
A^2	20.91	1	20.91	35.31	< 0.0001	
B^2	20.44	1	20.44	34.51	< 0.0001	
C^2	15.19	1	15.19	25.65	< 0.0001	
D^2	9.08	1	9.08	15.34	0.0011	
Residual	10.07	17	0.5921			
Lack of Fit	9.95	15	0.6635	11.70	0.0815	not significant
Pure Error	0.1134	2	0.0567			
Cor Total	140.20	26				

The Lack of Fit refers to the failure of a statistical model to adequately describe the relationship between variables in a dataset. This inadequacy could result from the model that is too simple or missing key variables, leading to discrepancies between the observed data and the model's predictions. The Lack of Fit *F*-value of 11.70 implied there was 8.15% chance that a Lack of Fit *F*-value this large could occur due to noise. In our model, Lack of Fit was not significant, that meant the model was compatible.

Table 4. Fit Statistics

Std. Dev.	0.7695	**R²**	0.9282
Mean	41.02	**Adjusted R²**	0.8902
C.V. %	1.88	**Predicted R²**	0.8188
		Adeq Precision	17.5207

The Predicted R² of 0.8188 was in reasonable agreement with the Adjusted R² of 0.8902; i.e. the difference was less than 0.2 (Table 4).

Adeq Precision measured the signal to noise ratio. A ratio greater than 4 was desirable. The ratio of 17.521 indicated an adequate signal.

The edible canna starch modification to form SDS using β-cyclodextrin was optimized with 4 influencing factors. The desirability was presented in Table 5.

Table 5. The desirability for optimization of SDS formation from edible canna starch using β -cyclodextrin

N°	Water content	β - CD content	Temp.	Time	SDS	Desirability
1	81.32	3.14	36.06	1.81	44.71	1.000
2	87.08	2.83	46.78	1.36	44.82	1.000
3	85.83	3.21	37.95	1.29	44.83	1.000
4	85.26	2.96	37.63	1.35	44.87	1.000
5	84.48	3.28	33.13	1.55	44.66	1.000
6	88.88	2.92	42.54	1.13	44.74	1.000

From Table 5, it was clearly seen that there were several solutions with an desirability of 1 to meet the purpose of maximizing the SDS starch content. However, in addition to the desirability, experimental conditions and easily adjustable parameters should be considered. Therefore, solution 1 with the following modification conditions: water content of 81.3%, β-CD content of 3.1%, temperature of 36 ºC, and time of 1.8 hours. With this modification parameters, the achieved SDS content was 44.714%.

Desirability of optimizing the SDS formation from edible canna starch was showed in Fig. 5. Fig. 5 indicated that the desirability was 1 when combining 4 factors, meaning that 100% achieved the maximum SDS content. Fig. 6 also indicated the optimizing parameters of the model as water content of 81.3%, β-cyclodextrin content of 3.1%, temperature of 36 ºC and time of 1.8h.

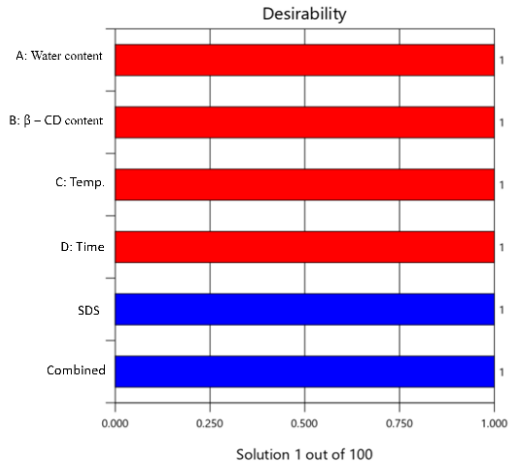

Fig. 5. Desirability of optimizing the SDS formation from edible canna starch

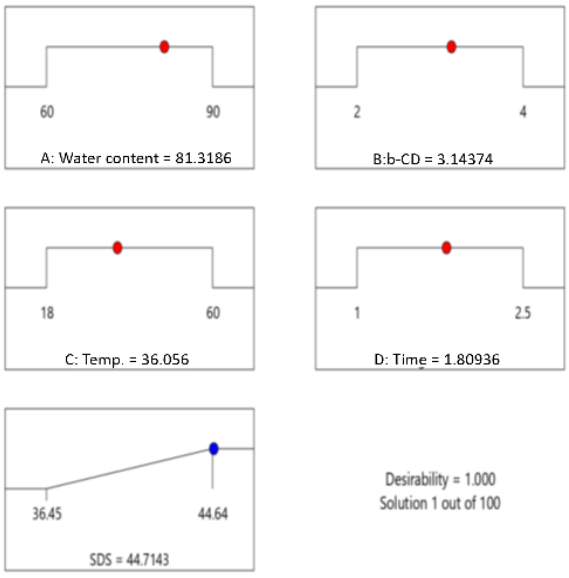

Fig. 6. The optimizing solution

To check the model compatibility, production of slow-digestible starch from edible canna starch was carried out according to the optimized process, after which the SDS content was determined and compared with the results predicted by the model. The results were shown in Table 6.

Table 6. Comparison of SDS content in edible canna starch produced by the optimized parameters with the values calculated by the model

	SDS content
Calculated from model	44.71
Determined by experiment	44.88 ± 0.90

From the results in Table 6, the modification conditions with a water content of 81.3%, β-CD content of 3.1%, reaction temperature of 36 ºC, and reaction time of 1.8 hours showed that the experimental results were consistent with the model predictions. Thus, the optimal conditions for the edible canna starch modification process using β-CD to gain the highest SDS content were:

- Water content 81.3%
- β - CD content 3.1%
- Temperature 36 ºC
- Time 1.8h

Here, since the results are primarily derived from the optimization algorithm, for example there was no evidence about structure of SDS and interaction between SDS and beta-CD…, therefore the discussions should be adjusted to align and relevant with the results about optimization.

4. Conclusion

Beta cyclodextrin affected the starch digestibility. β cyclodextrin could be the factor that modify edible canna starch to form slowly digestible starch (SDS). The optimal modification conditions to produce the highest amount of slow-digesting starch from cassava starch using β-cyclodextrin were as follows: water content of 81.3%, β - CD content of 3.1%, temperature of 36 ºC in 1.8 h. The digestible structure and properties of β-cyclodextrin modified edible canna starch would be interesting for our further studies.

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