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RESEARCH PAPER

Effect of hydrolysis of sweet potato starch by pullulanase enzyme on the formation of slowly digestible starch

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Abstract

The benefits of slowly digestible starch (SDS) are a slow or moderate increase in postprandial blood glucose levels and the maintenance of steady blood glucose levels. These created good conditions for diabetes control, satiety-hunger, physical and mental performance. Therefore, products related to slow digestion have attracted many researchers in recent years. In this research, technological factors affecting the formation of SDS from sweet potato starch (SPS) by the branching enzyme pullulanase were studied. These factors included SPS concentration, pullulanase enzyme concentration, pH, temperature, and hydrolysis time of pullulanase enzyme. The results showed that the maximum SDS yield was obtained by debranching for 5 hrs with an enzyme concentration of 20 ASPU/g, reaction temperature of 55°C, pH of 5.0, and a SPS concentration of 10% (w/w). In these conditions, the SDS content of SPS reached 28.04%. The findings indicated that sweet potato starch can be used as a raw material at a low cost and is feasible in the production of SDS which provides benefits for human health.

1. Introduction

Starch is a high molecular weight polysaccharide, containing hundreds to millions of glucose monomers which are linked to each other by α-glycosidic bonds. The starch also is the main carbohydrate in human nutrition. Starch digestibility in the human small intestine can be changed from rapid digestion to indigestibility. Thus, depending on the rate and extent of digestibility, starches have been classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). In the starch, there is a starch fraction with slow digestibility which benefits human health, named SDS. The SDS is digested at a slow rate in the small intestine through hydrolysis into glucose between 20 mins and 120 mins (Englyst *et al*., 1992). However, due to the amorphous, semicrystalline structure of starch, the digestive enzymes are difficult to access and function with starch, leading to a longer time for hydrolysis (Englyst *et al.*, 2003). The SDS helps improve blood glucose levels during and after meals. Also, the SDS has a positive effect on diabetes control, and prevention of metabolic diseases and cardiovascular complications and is helpful in controlling and preventing hyperglycemia-related diseases (Miao *et al*., 2015).

One of the main factors affecting the digestibility of starch is the ratio between amylose and amylopectin. The starch with high amylose content is difficult to digest and is used as a source of resistant starch. Meanwhile, the fully gelatinized glutinous starch is used as a source of rapidly digestible starch (Behall, 1988). This means that the starch contains high amylose content and is favored to produce SDS. Therefore, there have been some studies which have been focused on seeking methods to cleave amylopectin to increase the amount of amylose recently. Among the methods, using branched pullulanase enzyme in starch metabolism is of interest to researchers. The branching enzyme pullulanase acts on α-1,6 glycosidic bonds to cleave amylopectin to raise the amount of amylose (Lu *et al*., 2018). For example, Guraya *et al*. (2001) studied the effects of the branching enzyme pullulanase on glutinous rice starch and plain rice. The results showed that branching by the enzyme provided an opportunity for chains to link and reassemble to form crystals with a more perfect structure which in turn led to the formation of more SDS. In addition, the crystal arrangement in starch affected digestibility, with more

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digestible type A crystals, exhibiting higher amounts of RDS and SDS, and more indigestible type B crystals showing a higher amount of RS, the C-type starch indicating more resistance to enzymatic hydrolysis (Jane *et al.*, 1997). Cai and Shi (2014) showed that starch after hydrolysis by pullulanase tended to form type A and B crystals, which reduced starch digestibility.

Raw materials used for the production of SDS are mainly based on the starch of cereals. In Vietnam, the sweet potato is a popular root crop with high annual tuber production. However, sweet potatoes in Vietnam have been mainly used in the form of fresh tubers, so the value of agricultural products has not improved. The purpose of this study was to evaluate the effect of several factors, including sweet potato starch concentration, enzyme concentration, pH, temperature, and reaction time on the hydrolysis of sweet potato starch by pullulanase enzyme towards the formation of slowly digestible starch.

2. Materials and methods

2.1 Materials

Hoang Long sweet potato (summer-autumn crop) was collected in Viet Yen district, Bac Giang province. The sweet potato starch was obtained from grinding cleaned sweet potatoes. Pullulanase M2 derived from *Bacillus licheniformis* 2000 U/mL was provided by Megazyme. The α-Amylase from porcine pancreas A-3176 was purchased Sigma-Aldrich Chemical Co. (St. Louis, MO). The amyloglucosidase 3.260 U/mL E-AMGDF-40 mL, Invertase 2000 U/mL E-INVRT, D-Glucose Assay Kit (GOPOD Format) were achieved by Megazyme. Other chemicals used for adjusting pH, consisting of NaOH and H_2SO_4 were purchased from Sigma-Aldrich.

2.2 Isolation of sweet potato starch

First, the sweet potatoes were cleaned with water to remove dirt, and finely ground. Next, the mashed potatoes were sifted through the 60, 100 and 200 sieve, respectively, to obtain starch and remove the residue. The starch solution was left to settle for 6 hrs, removing the water and impurities on the surface of the settled starch. The settling process was repeated 3 times. Finally, wet starch was obtained and dried at 40°C until starch moisture was about 10%. Finally, the dried sweet potato starch was stored in a cool, dry place, avoiding insects and harmful animals and used for the next experiments.

2.3 Experimental

Effect of SPS concentration on the hydrolysis of SPS by pullulanase enzyme on the formation of SDS by varying concentrations of 5%, 7.5%, 10%, 12.5%, 15% (w/w) of SPS in acetate buffer at pH 4.0, 4.5, 5.0, 5.5, 6.0. The mixtures were then boiled and stirred for 30 mins then quickly reduced to the hydrolysing temperature, at which the 10, 20, 30, 40, 50 ASPU/g debranching pullulanase enzyme was supplemented into this mixture and let to further hydrolysis at 45, 50, 55, 60, 65°C and for 1, 2, 3, 4, 5, 6, 7, 8 hrs, respectively. The mixture was then boiled for 30 mins to stop the hydrolysis reaction. The hydrolyzed mixture was continuously centrifuged twice at 3000×*g* for 10 mins. The solid obtained was dried at 40°C for 12 hrs. The condition giving the highest SDS yield would be chosen for the next experiment respectively.

2.4 Digestibility of starch determination

The *in vitro* digestibility of SPS samples was determined according to the modified Englyst *et al*. (1992) method. The procedure was as follows: firstly, dissolving 200 mg of each branched starch sample in 15 mL of phosphate buffer (0.2 M, pH 5.2) by vortex. The slurry mixture was then heated stably at 37°C for 5 mins. The in vitro digestibility of starch slurry was determined by measuring the rate of starch hydrolysis by a digestive enzyme mixture of 54 mL pancreatic α-amylase enzyme solution (supernatant containing pancreatic α-amylase enzyme after centrifugation of 12 g in 80 mL water), 6 mL amyloglucosidase of 140 AGU/mL and 4 mL invertase of 2000 U/mL. After the solution was stabilized, 10 glass balls (2 mm in diameter) and 5 mL of the above digestive enzyme solution into the thermostat and the thermostat was continuously shaken at 150 rpm under 37^{o}C. At the time of 20 mins and 120 mins, using a mixture of 0.5 mL of hydrolyzate with 4 mL of absolute alcohol to inactivate the enzyme.

The amount of formed glucose was determined using the GOPOD assay kit. The percentage of hydrolyzed SPS was calculated as the concentration of formed glucose multiplied by 0.9.

The obtained RDS, SDS, and RS ratios were calculated by the following equations:

$$\%RDS = (G20 - FG) \times 0.9 \times 100 \quad (1)$$

$$\%SDS = (G120 - G20) \times 0.9 \times 100 \quad (2)$$

$$\%RS = (TG - FG) \times 0.9 \times 100 - \%RDS - \%SDS \quad (3)$$

Where, G20 and G120 are the total glucose content released after 20 mins and 120 mins, respectively; FG is free glucose; TG is total glucose.

2.5 Statistical analysis

The experimental data were analyzed using

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Microsoft Excel and SPSS Statistic 20 for Windows software. The difference in all experiments is $P < 0.05$. All experiments were in triplicate. The results were expressed as average value ± standard deviation.

3. Results and discussion

3.1 Effect of sweet potato starch concentration on digestibility of sweet potato starch hydrolyzed by pullulanase enzyme

The effect of SPS concentration on the hydrolysis of SPS by pullulanase enzyme towards the formation of SDS was illustrated in Figure 1. Figure 1 shows that the hydrolysis degree of SPS to form SDS increased when the SPS concentration increased from 5 to 10%. The maximum SDS yield was 27.68% at SPS concentration of 10% (w/w). The SDS yields were 19.26% and 25.96% when SPS concentrations were 5% and 7.5%, respectively. The results were due to an increase in the substrate concentration causing a growth in enzyme activity rate. Besides, at the initial stage of the reaction, the rate of the reaction was almost proportional to the substrate concentration leading to more substrate molecules bound to the enzyme molecules, thus the formed products were promoted (Kuddus, 2019). This result was similar to that observed by Kuddus (2019). Similarly, according to Zeng *et al.* (2015), the hydrolysis efficiency of glutinous rice starch by pullulanase enzyme also gave the highest SDS content (29.6%) at 10% starch concentration.

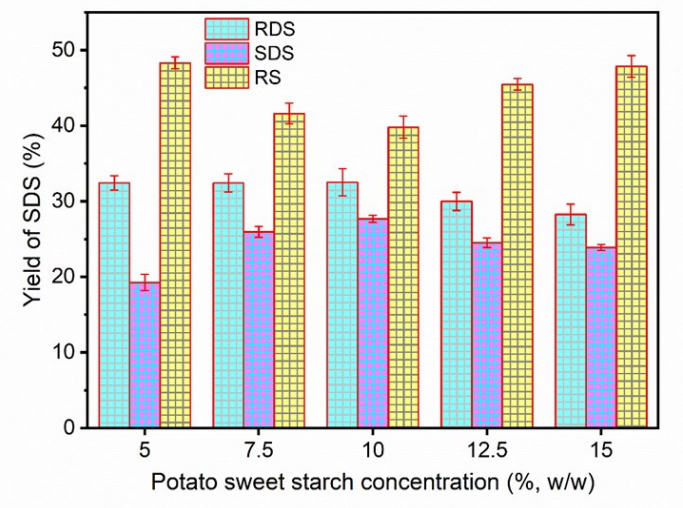

Figure 1. Effect of SPS concentration on digestibility of SPS hydrolyzed by pullulanase.

However, the hydrolysis degree of SPS to produce SDS decreased slightly when the SPS concentration was above 10% (w/w). Specifically, at SPS concentrations of 12.5% and 15%, the SDS yield was low and reached 24.52% and 23.88%, respectively. The reason for the decrease in SDS yield was that the water acted as both the reaction and transport medium in the enzymatic hydrolysis reaction which was beneficial for diffusion and movement of molecules in the reaction, so substrate and enzyme were evenly distributed and better contact. Besides, the water also helped SPS to disperse well and prevented a partially high concentration of products in the mixture, which inhibited the hydrolysis reaction (Ferreira and Hultin, 1994). Su and Cheng (2010) indicated that a high starch concentration results in a poor swelling capacity. As a result, the interaction between enzyme and substrate was significantly decreased. Therefore, the formation of SDS is less efficient (Su and Cheng, 2010). Thus, the SPS concentration of 10% (w/w) was chosen for the next hydrolysis process.

3.2 Effect of enzyme concentration on digestibility of sweet potato starch hydrolyzed by pullulanase enzyme

The effects of enzyme concentration on the digestibility of SPS hydrolyzed by pullulanase enzyme are presented in Figure 2. As can be seen from Figure 2, the change in yields of RDS, SDS, and RS strongly depended on the change in the concentration of the pullulanase enzyme. When the concentration of pullulanase enzyme increased from 10 ASPU/g to 50 ASPU/g, the content of RDS and SDS gradually increased while RS yield decreased gradually. Specifically, RDS content increased from 29.89% to 38.73%, SDS content rose from 20.55% to 28.75%, and RDS decreased from 49.56% to 32.52% in accordance with a rise in pullulanase enzyme concentration from 10 ASPU/g to 50 ASPU/g. The results could be explained that the presence of the pullulanase enzyme led to the cleavage of the amylopectin branched chain of SPS which created many amylose-like chains. These chains were converted into a double helices structure after the retrogradation stage, making the starch structure more stable which resulted in preventing the action of digestive enzymes. The increase in yields of SDS and RS strongly depended on the length of the double helices and the way of retrogradation. The chains with a low

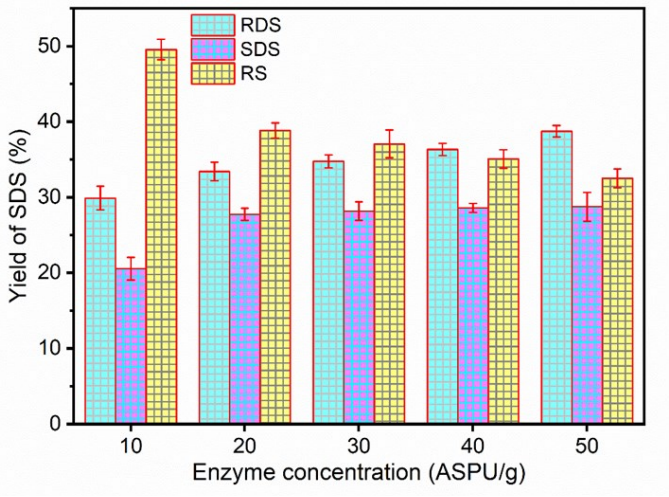

Figure 2. Effect of enzyme concentration on digestibility of SPS hydrolyzed by pullulanase.

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degree of polymerization (DP), the main product formed an amorphous region (SDS). Meanwhile with longer DP, the possibility of production of RS crystalline product was higher (Babu and Parimalavalli, 2018). Statistical analysis results also showed that when increasing enzyme concentration from 20 ASPU/g to 50 ASPU/g, SDS content did not change. Therefore, the optimal amount of pullulanase was chosen to be 20 ASPU/g for the next hydrolysis reactions. Babu and Parimalavalli (2018) produced SDS from sweet potato starch with 2% pullulanase after autoclaving at 120°C/30 mins and followed by storage at 4°C the best technique to produce a high amount of RS (28.76%) and at 32°C for a high amount of SDS (20.9%).

Besides that, Wu *et al*. (2009) indicated that the optimal amount of pullulanase was 10 ASPU/g. Zhang *et al*. (2011) reported that the appropriate pullulanase content for SDS production from maize starch was 12 ASPU/g. Miao *et al*. (2009) showed that the highest SDS content could be obtained by enzyme pullulanase hydrolysis on waxy maize starch at high concentrations (20 or 40 ASPU/g) branched for 3–6 hrs (Miao *et al*., 2009). There was a difference in the optimal concentration of pullulanase used in hydrolysis reactions for SDS production in various publications may be due to differences in starch origin (Zhang *et al.*, 2011)

3.3 Effect of pH on digestibility of sweet potato starch hydrolyzed by pullulanase enzyme

pH plays an important role in the hydrolysis reaction of starch by enzymes to produce SDS. In this study, the effect of pH on the formation of SDS from SPS using pullulanase enzyme was studied and the results are shown in Figure 3.

It can be seen from Figure 3 that the highest SDS yield was 27.73% which was obtained at pH of 5.0. While the SDS yield was the lowest at pH 6.0 (19.38%).

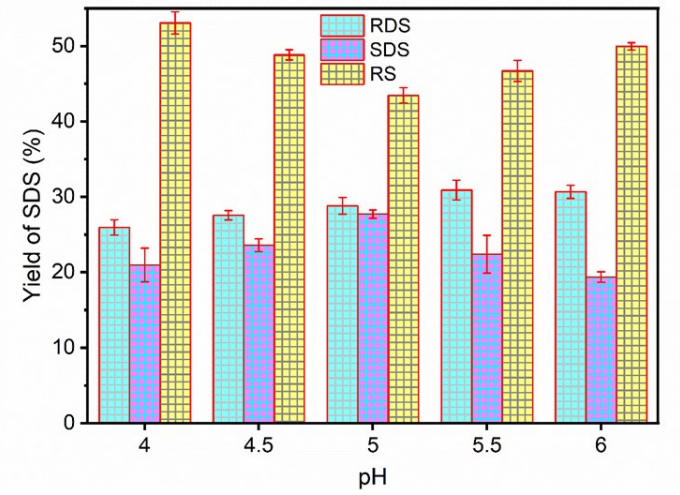

Figure 3. Effect of pH on digestibility of SPS hydrolyzed by pullulanase.

Besides, the statistical analysis results showed that the SDS content obtained in this hydrolysis reaction at pH 5.0 was the highest and had a significant difference compared with the SDS content obtained at other pH values. These results were similar to previously reported results. For example, the optimal pH used in the hydrolysis of various starches by the pullulanase enzyme was in the range of 5.2 to 5.5 (Lu *et al*., 2018).

Based on the kinetics of the enzymatic reaction, the active site of enzyme catalysis containing several charged amino acid groups on the side chains, which bound to the substrate and convert the substrate to product, must be in a particular state of dissociation. The pH affected the enzyme and the dissociation state in the substrate, which in turn affected the stability of the enzyme, the ability to bind the enzyme to the substrate, and the conversion of the substrate to the product, and thus affected the catalytic action of the enzyme (Clemente *et al*., 1999). Thus, pH 5.0 was chosen for conducting the next SPS hydrolysis experiments.

3.4 Effect of temperature on digestibility when hydrolyzed by pullulanase

Temperature plays a vital role in enzyme-catalyzed reactions. Pullulanase is a thermostable enzyme that works optimally in the temperature range of 45-65°C. Thus, this temperature range was also chosen to investigate the effect of temperature on the hydrolysis of SPS for the formation of SDS. The obtained results are indicated in Figure 4.

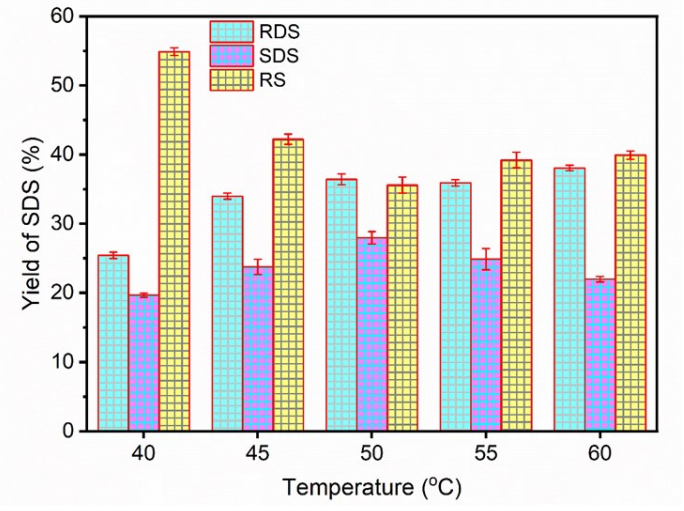

Figure 4. Effect of temperature on digestibility of SPS hydrolyzed by pullulanase.

The results in Figure 4 indicated that the hydrolysis capacity of the pullulanase enzyme increased in the temperature range from 45°C to 55°C and peaked at 55° C with the maximum SDS content of 27.98%. However, with a further increase from 55°C to 65°C, the SDS content of starch decreased. This could be explained by the fact that at temperatures below 55°C, the enzyme

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activity was lower, thus the hydrolysis rate was low. When the temperature was up to 55°C, the peptide bonds could bind easily, so the hydrolysis rate was increased. In contrast, when the temperature was too high, the enzyme became inactivated, thus the reaction slowed down, leading to a low SDS yield (Shu *et al*., 2016). The pullulanase enzyme could hydrolyze at high temperatures, but the optimal temperature for hydrolysis also varied depending on the type of substrate and the source of the enzyme (Simsek *et al*., 2012) hydrolyzed starches by pullulanase enzyme at 60°C or Zhang *et al*. (2019) hydrolyzed starch for the highest SDS yield by the pullulanase enzyme at 50°C. A temperature of 55°C was selected for the next experiment.

3.5 Effect of hydrolysis time on digestibility when hydrolyzed by pullulanase

The results of studying the effect of reaction time in the range from 1 to 8 hrs on the SPS hydrolysis by pullulanase enzyme are presented in Figure 5.

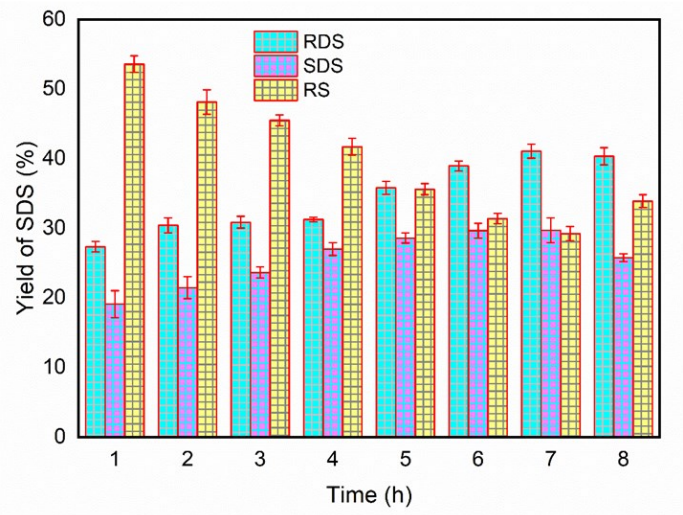

Figure 5. Effect of temperature on digestibility of SPS hydrolyzed by pullulanase.

From Figure 5, it is clearly seen that there was a gradual growth in formed SDS yield when reaction time increased from 1 to 6 hrs. The content of formed SDS was the highest with 29.64% after 6 hrs of hydrolysis. The lowest produced SDS yield was 19.09% after 1 hr of reaction time. This can be explained that when the hydrolysis time increased, the enzyme had more chance to contact the substrate and thus enhanced the hydrolysis efficiency, leading to the formation of more SDS. However, there was no difference in the formed SDS yield between 5 to 7 hrs. Specifically, the SDS yields reached 28.59%, 29.64%, and 29.70%, respectively, at hydrolysis time of 5, 6 and 7 hrs. The results were because of a prolonging in reaction time leading to a gradual decrease in substrate concentration. Besides, the reaction site was saturated by enzyme molecules and formed strong competitive inhibition with the product (Shu *et al*., 2016). The SDS content decreased to 25.75% at 8 hrs of reaction time. This can be caused by the formation of short-chain soluble molecules since the pullulanase enzyme is a completely non-specific enzyme that can cleave α-1,4 glycoside bonds (Singh and Singh, 2019). The research results are also consistent with the assertion of Miao *et al*. (2019) who reported hydrolysis time of waxy corn starch by pullulanase enzyme changed from 1 to 24 hrs, the SDS content was highest when the hydrolysis time of pullulanase was in the range of 3-6 hrs and tended to decrease as the hydrolysis time increased up to 24 hrs.

4. Conclusion

In summary, in this study, the SDS product was successfully produced from sweet potato starch. The effect of various factors on the hydrolysis reaction of SPS by pullulanase enzyme for the production of SDS was systematically studied. The optimal parameters for the hydrolysis of sweet potato starch under the action of pullulanase enzyme were selected, including SPS solution concentration of 10% (w/w), pH of 5.0, temperature of 55°C, pullulanase enzyme concentration of 20 ASPU/g, and hydrolysis time of 5 hrs.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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