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Modification of hydrolyzed sweet potato starch by retrogradation to enhance slowly digestible starch yield

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Abstract

Recently, slowly digestible starch (SDS) and resistant starch (RS) have attracted the great attention of consumers and the food industry due to their beneficial effects on human health. The SDS ingestion resulted in a prolonged release of glucose to maintain the glycemic index (GI) from low to moderate levels. Thus, consumption of SDS might help fully control and prevent hyperglycemia-related diseases, such as cardiovascular disease and diabetes. In this study, the sweet potato starch hydrolyzed by pullulanase enzyme was continuously processed by dual-retrogradation to enhance the formation of SDS. SDS was determined by the analytical method developed by Englyst. Results showed that in the first retrogradation cycle, the obtained SDS content was 44.76±0.37% at 4°C after 48 h of reaction time. The formed SDS content reached 59.72±0.97% at 4°C after 48 h of reaction time in the second retrogradation cycle. As a result, in the whole retrogradation process, the SDS content from sweet potato starch saw a remarkable increase from 15.04% to 59.72%.

1. Introduction

Sweet potatoes (*Ipomoea batatas* (L) Lam) are ranked as one of the most important food crops, followed by rice, wheat, potato, maize, and cassava (Wang *et al.*, 2016). According to Food and Agriculture Organization statistics, 113 countries have been growing sweet potatoes around the world with an annual production of about 103 million tons (Ngoc *et al*., 2017). The components of sweet potato include carbohydrates (mainly starch), vitamins, minerals, and fiber. Among them, the starch plays an important role in the human diet because it provides 70-80% of daily energy consumption (Ramesh Yadeav *et al*., 2006).

Based on the rate and extent of starch digestion *in vitro*, starch is often classified into three types, rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst *et al*., 1992). The RDS is a part of starch that is digested within 20 mins, the SDS is the portion digested after 20 mins to 120 mins, and the RS is the remaining starch after 120 mins of digestion. Ingestion of foods containing SDS resulted in a low to moderate glycemic index (GI), opposite to a high GI from foods containing RDS (Englyst *et al.*, 2003). Hence, SDS consumption can help control and prevent diseases related to hyperglycemia, such as cardiovascular disease and diabetes (Miao *et al*., 2013).

Previous studies indicated various strategies for starch modification to improve SDS content, such as physical methods, including retrogradation (Guraya *et al*., 2001; Shin *et al*., 2004; Hu *et al*., 2014), heat-moisture treatment (Lee *et al*., 2012; Huang *et al*., 2016; Cahyana *et al*., 2019), electric field treatment (Zeng *et al*., 2016), chemical methods such as acid treatment (Shin *et al.,* 2007), cross-linking (Wolf *et al*., 1999), oxidation (Wolf *et al*., 1999), and enzyme methods using pullulanase (Miao *et al*., 2009; Lu *et al*., 2018), amylase (Shin *et al*., 2004). Among these methods, the retrogradation after debranching by pullulanase is an effective and safe method. The debranching starch by pullulanase dislodges a-1,6 linkage, which contributes to the increase of amylose content and short linear dextrins. This can lead to an increase in crystallinity, which is associated with the formation of SDS (Liu *et al*.,2017). Starch retrogradation is an inevitable phenomenon during the storage of food in which gelatinized starch is transformed from an amorphous state to a more ordered structure or crystalline one (Tian *et al*., 2009; Babu *et al.*,

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2018). SDS and RS formation during processing related to retrogradation process and the trend in SDS yield is as a function of pullulanase content, storage temperature, autoclave cycle and their mutual interaction (Lee *et al*, 2013). In the retrogradation period, amylose and amylopectin can be rearranged or form new bonds among starch molecules in a crystalline area after the disruption of the starch structure in the gelatinization (Hu *et al*., 2014; Babu and Parimalavalli, 2018). The result is the formation of imperfect crystallites, which compose the fundamental structure of SDS (Hu *et al*., 2014). The increase in amylose content has been advantageous for the formation of imperfect crystallites and amorphous regions in retrogradation (Tian *et al*., 2009). Different conditions for retrogradation after debranching starch by pullulanase were reported. Guraya *et al*. (2001) debranched rice starch by pullulanase combined with retrogradation and reported that the optimum retrogradated temperature after debranching was 1°C that could produce highest amount of SDS (Guraya *et al*., 2001). Babu *et al*. (2018) found that storing pullulanase hydrolysed sweet potato starch at 4°C led to a higher degree of retrogradation than storing at higher temperatures (30°C and 60°C). 4°C is the temperature that formed a maximum SDS from pullulanase debranched waxy sorghum starch for 72 hrs (Shin *et al*., 2004), from pullulanase debranched waxy corn starch for two days (Miao *et al*., 2009). Freezing/thawing treatment resulted in higher RDS and SDS contents and lower RS content compared to native waxy rice starch (Tao *et al.*, 2015)

In this study, the effect of the single-, dual- and triple -retrogradation conditions after debranching of SPS by pullulanase enzyme on SDS content were studied.

2. Materials and methods

2.1 Materials

In this study, Hoang Long sweet potato (summer-autumn crop) collected in Viet Yen district, Bac Giang province was used as a raw material to produce sweet potato starch. All enzymes, including Pullulanase M2 derived from *Bacillus licheniformis* 2000 U/mL, amyloglucosidase 3260 U/mL E-AMGDF-40 mL, Invertase 2000 U/mL E-INVRT, and D-Glucose Assay Kit (GOPOD Format) were provided by Megazyme. α-Amylase from porcine pancreas A-3176 was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

2.2 Isolation of sweet potato starch

First, the sweet potato roots were cleaned with water to remove dirt, and finely ground. Next, the ground sweet potatoes were sifted through the 60, 100 and 200 sieves, respectively, to obtain starch and remove the residue. The starch solution was left to settle for 6 hours, removing the water and impurities on the surface of the settled starch and the settling process was repeated 3 times. Finally, wet starch was obtained and dried at 40^{o}C until the moisture content was approximate 10% The sweet potato starch was finally packed and stored in a cool and dry place to avoid the attack of insects and harmful animals. This SPS was used for further experiments.

2.3 Preparation of slowly digestible sweet potato starch

The sweet potato starch slurry (10% w/v dissolved in acetate buffer 5.0) was boiled and stirred for 30 mins. The samples were cooled until the temperature reached 55°C. The samples were then debranched by adding pullulanase enzyme at a concentration of 20 U/g. The hydrolyzing was carried out at 55^{o}C for five hours. Immediately after the reaction, the solutions were boiled for 30 mins to inactivate the enzyme and terminate the hydrolysis reaction. Then, the mixture was hermetically sealed and stored at -20°C, -10°C, 4°C, 25°C for about 12-60 hrs for the single-retrogradation. The single-retrograded starch samples were then boiled for 30 mins and continued to perform the same retrogradation. Under the same conditions as abovementioned, the sweet potato starch was followed by heating to perform single-, dual-triple-consecutive retrogradation cycles. Afterward, the mixture was centrifuged three times and cleaned with distilled water. The obtained modified starch was dried at 40°C for 24 hrs to a moisture content of less than 10% and milled to pass through a 120-mesh sieve. The obtained SPS was used to analyze SDS content.

2.4 Slowly digestible starch, rapidly digestible starch and resistant starch determination determination

The digestibility of SPS was determined according to the method of Englyst *et al*. (1992) and Miao *et al.* (2014) with a minor modification (Englyst *et al*., 1992; Miao *et al.*, 2014). The analysis procedure was described in detail as follows: firstly, the used enzyme solution was a mixture of 54 mL pancreatic α-amylase enzyme solution which was prepared by diluting 12 g of pancreatic α-amylase enzyme in 80 mL of water, magnetically stirring for 10 mins, then centrifuging for 10 mins at 1500×*g*, and decanting 54 mL of supernatant after centrifugation, 6 mL amyloglucosidase solution 140 AGU/mL, and 4 mL invertase 2000 U/mL. Then, 200 mg of SPS was dissolved in 15 mL of phosphate buffer (0.2 M, pH 5.2) with vortex. The SPS slurry was equilibrated in a water bath for 5 mins at 37°C. The SPS slurry was then added 10 glass balls of 2 mm in diameter and 5 mL of prepared enzyme solution. The reactor was continuously agitated at 150 rpm under 37°C. Afterwards, 0.5 mL of hydrolysate was removed at 20

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and 120 mins, and 4 mL of absolute alcohol was added to each tube to inactivate the enzyme.

The formed glucose content was analyzed by the GOPOD assay kit. The percentage of hydrolyzed SPS was computed as the concentration of formed glucose multiplied by 0.9.

The resulting RDS, SDS, and RS yields are computed using 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, RDS is rapidly digestible starch; SDS is slowly digestible starch; RS is resistant starch; FG represents free glucose content (g); TG is total glucose (g); G20 is glucose content released after 20 mins (g) and G120 expresses glucose content released after 120 mins (g).

2.5 Statistical analysis

In this study, all experiments were repeated in triplicate. Analysis of variance was performed using Ducan's multiple-range test using Microsoft Excel and SPSS Statistic 20 software at the 5% level ($P < 0.05$). The results were described as average mean value ± standard deviation (SD).

3. Results and discussion

3.1 Effect of single-retrogradation treatments on the slowly digestible starch content

3.1.1 Temperature

The experiment to investigate the effect of the single -retrogradation temperature on the SDS content was conducted for 36 hrs of reaction time with varying reaction temperatures at -20°C, -10°C, 4°C and 25°C. The obtained results were presented in Table 1.

Table 1. Effect of single-retrogradation temperature on the SDS content.

Single-retrogradation temperature (°C)	SDS content (%)
-20°C	36.85±1.54[a]
-10°C	37.18±0.57[a]
4°C	44.50±0.49[b]
25°C	37.89±0.82[a]

Values are presented as mean±SD of triplicate analyses. Values with different superscripts are statistically significantly different ($p<0.05$).

The data in Table 1 showed that the highest SDS content was 44.50% with starch samples degraded at 4°C and the lowest SDS content was 36.85% at 20°C. However, there had no significant difference about yield of SDS at a temperature of -20°C, -10°C and 25°C. The difference in obtained SDS contents at various retrogradation temperatures were explained that the decrease of starch gel temperature after gelatinization and enzymatic hydrolysis led to the crystallization (retrogradation) of the starch. After enzyme treatment and storage, sweet potato starch structure changed to a combination of B and V types (Babu *et al*., 2018). The structures destroyed after the hydrolysis process which increased the number of linear chains or less branched or lower molecular mass were recrystallized to form imperfect crystals which resulted in the slowly digestible of starch (Guraya *et al*., 2001; Babu *et al*., 2018).

According to Morris (1990), the formation process of a crystal includes 3 steps: (1) nucleation – formation of crystal nuclei; (2) propagation–growth of crystals from sprouts and (3) maturation–the completion of the crystal or the slow growth of the crystal. The process of crystal nucleation occurred at a cold temperature (4°C). At that time, the formed crystal was incomplete, so enzyme attack could still take place but at a slow rate, leading to the high SDS content. The process of forming mature crystals occurred when retrogradation of starch took place at room temperature (25°C). At this condition, a complete crystal was very difficult to be broken down, so the content of resistant starch (RS) was the highest (Morris, 1990; Guraya *et al*., 2001).

Guraya (2001) studied retrogradation on rice starch and showed that keeping starch after hydrolysis by pullulanase at 1°C facilitated the formation of SDS, while the temperature of 15°C produced more RS (Guraya *et al*., 2001). Similarly, Shin *et al*. (2004) studied the production of SDS from waxy sorghum starch and concluded that the temperature for the highest yield of SDS was 1°C. For waxy corn starch, retrogradation at 4°C affected the ratio of SDS to RS and RDS due to increased nuclei number during recrystallization which opposed to propagation and maturation steps, retrogradation at this temperature rose the SDS content up to 45.1% (Shin *et al*., 2004; Miao *et al*., 2009; Liu *et al*., 2017). Retrogradation during storage at 4°C for 4 days produced the highest SDS of high hydrostatic pressured-treated waxy wheat starch (Hu *et al*., 2017) or for 7 days changed the structure of starch, reduced retrogradation and enhanced slowly digestible starch (Ji *et al*., 2022). An excellent level of RS has been shown in storing debranched sweet potato starch at lower and higher temperatures, otherwise an increased level of SDS was seen at a moderate holding temperature (Babu *et al*., 2018). Li *et al*. (2020) observed the changes of 4°C retrograded starch after microwave treatment and found that the microwave treated rice starch displayed stronger molecular reorganizations and

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higher SDS content than untreated one. The temperature of 4°C was the suitable temperature for single-retrogradation of SDS from SPS.

3.1.2 Time

During single retrogradation, there is the recrystallisation of disordered crystallite structures to form imperfect crystallites, resulting in an increase in the amount of SDS (Hu *et al*., 2014). So, in this section, the effects of single-retrogradation time were studied and the results were shown in Figure 1.

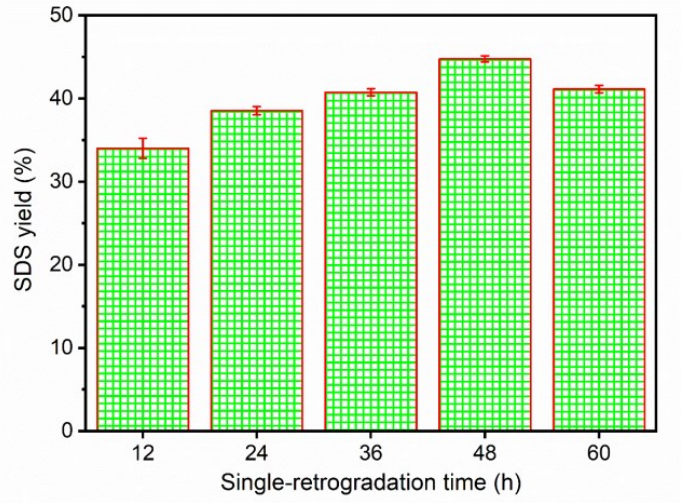

Figure 1. Effect of single-retrogradation time on the SDS content.

As shown in Figure 1, the maximum SDS content reached 44.67% when degraded sweet potato starch gel in 48 h. Meanwhile, SDS content reached the lowest when retrograded in 12 hrs. The data in the Figure 1 revealed that there had no linear correlation between obtained SDS content with the retrogradation time and a moderate retrogradation was sufficient to increase the SDS contents (Zhang *et al*., 2011).

This can be explained by the fact that at the beginning of the retrogradation process, the crystallite structure which was disordered after being hydrolyzed by the pullulanase enzyme became more ordered to form imperfect crystals. Normally, free amylose was the important factor that support in the crystalline region of starches and in this case, the short linear glucans formed from the pullulanase hydrolysis of starch could increase the crystallites of starches (Liu *et al*., 2017). This led to an increase in the yield of SDS, a fall in RDS content and an unchanged in RS content because almost RDS was converted into SDS. At the later retrogradation period (after 48 hrs), the RS content started to increase due to the increasing ordered crystal structure after a certain time; meanwhile, the amount of SDS decreased and there had no significant change in obtained RDS yield because the SDS was gradually converted into RS (Hu *et al.,* 2014).

The similar results were also achieved by other publications. For instance, Zhang *et al*. (2011) studied retrogradation of SDS on rice starch and waxy rice and showed that the content of SDS was not linearly dependent on the retrogradation time. The SDS content on waxy rice starch was the highest after 7 days of retrogradation with 51.62% (Zhang *et al*., 2011). For rice starch, Tian *et al*. (2013) similarly concluded that the retrogradation with suitable time facilitated the production of SDS, 36 hrs was chosen time for this process (Tian *et al*., 2013). Ming Miao *et al*. (2009) studied the effect of retrogradation time after waxy corn starch hydrolysed by pullulanase enzyme on the content of slowly digestible starch. The results reported that the rise in retrogradation time was not proportional to the increase in SDS content. The retrogradation time for the highest SDS content from waxy corn starch was 48 hrs. After this time period, there was no increase in obtained SDS content (Miao *et al*., 2009). Lu *et al*. (2018) demonstrated that it should be autoclave the retrograded debranched pea starch gels to increase SDS content and indicated that when retrogradation time of debranched pea starch gel increased to 14 days, SDS content increased to 7%. The retrograded amylopectin and its thermal reversibility during cold storage was the main factors for SDS to be formed (Lu *et al.*, 2018). Similarly, branching starch by enzyme from *Bifidobacterium longum* (BlBE) reduced amylose content, rose short-branch chains proportion and number of α-1,6 branching points in amylopectin, thus obviously increased the SDS content of wheat noodles (Li *et al*., 2020; Li *et al*., 2022). So that, the appropriate time for the single-retrogradation of SPS was 48 hrs.

3.2 Effect of dual-retrogradation treatments on the SDS content

After the single-retrogradation at temperature of 4°C in 48 hrs, the sweet potato starch continued to be retrograded for the second time.

3.2.1 Temperature

The dual-retrogradation experiment of sweet potato starch was carried out at the following temperatures: -20°C, -10°C, 4°C and 25°C within 48 hrs of reaction time. The results were presented in Table 2.

Table 2 showed that the SDS content was highest (59.09%) at 4°C. The lowest SDS content was 46.09% at -20°C. There was a good correlation between obtained SDS content in single-retrogradation and dual-retrogradation at various retrogradation temperatures. SDS content. This could be explained similarly as the single-retrogradation process, the crystal nucleation process occurred at cold temperatures (4°C), leading to

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the formation of more incomplete crystals. In this process, the formed crystal can be attacked by the digestive enzyme at a slow rate (Guraya *et al.*, 2001). The retrogradation at 4°C promoted the formation of crystal nucleation but inhibited propagation and maturation because the propagation was a diffusion phenomenon that could be controlled and would be close to zero at refrigeration temperature (Guraya *et al.*, 2001). Meanwhile, the mature crystal formation process occurred when the SDS was retrograded at room temperature (25°C). And because the complete crystal was very difficult to be decomposed, thus the content of resistant starch (RS) was higher at that temperature (Slade and Levine, 1987; Morris, 1990; Guraya *et al.*, 2001). That was the reason why at 25°C, the SDS content was lower than that of at 4°C. This was because the imperfect crystallites (SDS) had matured to form perfect crystals or a part of the SDS had been converted into RS.

Table 2. Effect of dual-retrogradation temperature on the SDS content.

Dual-retrogradation temperature (°C)	SDS content (%)
-20°C	46.09±0.70[a]
-10°C	46.79±0.16[a]
4°C	59.06±0.46[b]
25°C	46.63±1.41[a]

Values are presented as mean±SD of triplicate analyses. Values with different superscripts are statistically significantly different ($p<0.05$).

Moreover, Slade *et al.* (1987) reported that the crystal nucleation rate approached 0 at T_m (temperature of melting) and maximum at T_g (glass transition temperature) while the crystal growth rate approached 0 at glass transition temperature but reached a maximum at melting temperature. The actual rate of crystallization (nucleation and growth) would have a maximum value at the temperature of $T = 1/2 (T_g + T_m)$, which was usually close to room temperature (Slade and Levine, 1987).

Compared with the single-retrogradation, the dual - retrogradation at the same temperature gave a significantly higher content of SDS. At 4°C the SDS content was increased from 44.50% in the single-retrogradation to 59.06% in the dual-retrogradation and at 25°C, the SDS content grew from 37.89 to 46.63%. Similarly, the content of SDS at -20°C increased by 9.24% and at -10°C increased by 9.61%. The higher SDS content in dual - retrogradation was probably due to the formation of more imperfect crystals, including short crystalline chains and amorphous clusters (Robin *et al.*, 2008) and imperfect crystals structure of SDS (Zhang *et al.*, 2011). A similar result was achieved by Tian *et al.* (2013) who studied the effect of single-, dual-retrogradation treatment on rice starch and concluded that the SDS contents in rice starch treated with dual-retrogradation treatment (56.7%) was higher than that of the single-retrogradation treatment under the same conditions (39.3%). So, the suitable temperature for dual - retrogradation of SPS to gain high SDS content was 4° C.

3.2.2 Time

The effect of dual-retrogradation time on the digestibility of SDS was performed at 4°C during 12-60 hrs. Figure 2 showed that the amount of obtained SDS gradually increased from 45.76% during 12 hrs to 59.72% during 48 hrs. There was a slight decrease in SDS content of 55.51% at 60 hrs of reaction time. These results were in accordance with the results of Tian *et al.* (2013). Tian et al found that the dual retrogradation treatment significantly increased the SDS of high-level amylose rice starch after retrogradation for 36 hrs and reduced slightly when retrograding for longer time. This may be due to the dual retrogradation of high evel amylose rice starch could create higher amount of amylose-lipid complexes (Tian *et al.*, 2013) than that from sweet potato starch, which occupied the lower amount of amylose. And the moderate retrogradation time of 48 hrs could increase the amount of formed slowly digestible starch (Zhang *et al.*, 2011). Hu *et al.* (2014) reported that the yield of slowly digestible starch with retrogradation time interval of 48 hrs grew up to 44.41%. Thus, the appropriate time for the dual-retrogradation of sweet potato starch in this study was 48 hrs.

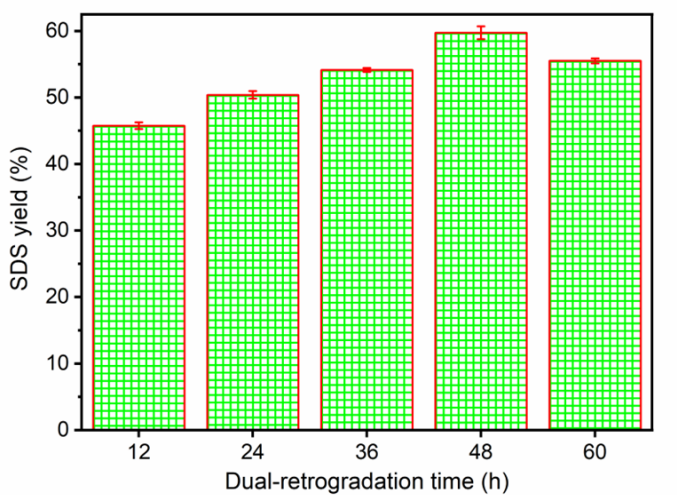

Figure 2. Effect of dual-retrogradation time on the SDS content.

3.3 Effect of triple-retrogradation treatments on the SDS content

After single- and dual-retrogradation at 4°C in 48 hrs, the SDS continued to retrograde for the triple-retrogradation. The triple-retrogradation was carried out

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a 4°C during a time period from 12 hrs to 60 hrs. The effects of triple-retrogradation on SDS content were presented in Figure 3.

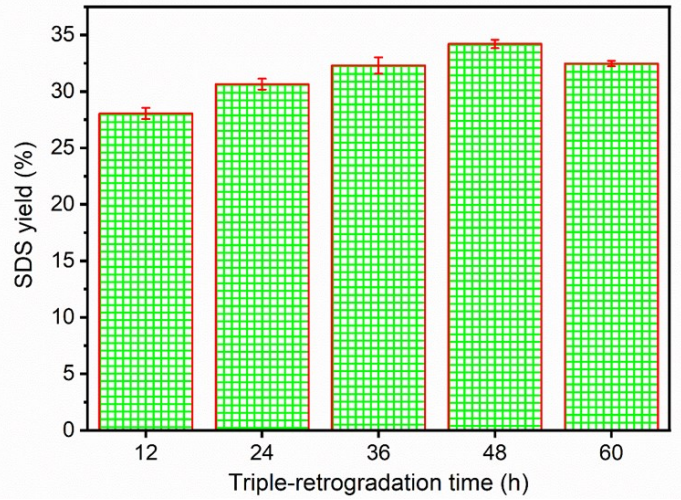

Figure 3. Effect of triple-retrogradation time on the SDS content.

The data in Figure 3 showed that the SDS content obtained by the triple-retrogradation was remarkably decreased in comparison with the SDS content obtained by dual-retrogradation at the same conditions. After 48 hrs of retrogradation, the SDS content fell from 59.72% in dual-retrogradation to 34.21% in triple-retrogradation. The decrease in SDS content in triple-retrogradation could be due to the breaking of double helices, resulting in the formation of starch crystals on the grain's surface or a reorientation of crystals (Chung *et al*., 2009). The increase in RS content and the decrease in SDS content during the modification showed the partial conversion of SDS into RS. Jagannadham *et al*. (2017) found that the amount of SDS contents of chickpea starch after triple-retrogradation was considerably decreased from 52.05% to 26.31% for 48 hrs of retrogradation time (Jagannadham *et al*., 2017). Besides, single-, dual- and triple-retrogradation processes affecting the SDS content obtained from glutinous wheat starch were also reported by Hu *et al*. (2014). After the same retrogradation time of 48 hrs, the yield of SDS obtained from the triple-retrogradation was lower than that of dual-retrogradation (from 44.41% to 38.04%) (Hu *et al*., 2014). Thus, it can be concluded that the triple-retrogradation process of the starch hydrolyzed by the enzyme pullulanase was not favorable for the production of SDS. Hence, triple-retrogradation of SPS after enzymatic hydrolysis by pullulanase was not favorable for SDS production.

3.4 Slowly digestible content of modified sweet potato starch

Based on the parameters chosen above, SDS from SPS was produced as followed: 10% (w/v) sweet potato starch was hydrolyzed at pH 5.0 with acetate buffer, enzyme concentration 20 U/g, temperature 55°C for 5 hrs, after that single – retrogradation at 4°C in 48 hrs was 44.76%, dual-retrogradation at 4°C in 48 hrs was 57.82%. The SDS content of native and modified SPS are presented in Table 3.

Table 3. SDS content obtained from raw and modified starch after the dual-retrogradation process.

	Natural sweet potato starch	Modified starch
SDS (%)	15.41±0.90	57.82 ±1.21

The results from Table 3 showed that the SDS content obtained by starch hydrolyzed by pullulanase enzyme and followed by dual-retrogradation was remarkably increased by 57.82%. This result was similar what obtained by Tian *et al*. (2013) who studied the formation of SDS in rice starch after modified by dual-retrogradation with a high content of SDS (56.7%).

4. Conclusion

In conclusion, to increase the SDS content for the sweet potato starch after hydrolyzed by enzyme pullulanase, a retrogradation step should be carried out. When retrograded hydrolyzed SPS at 4°C for 48 hrs, the SDS content reached the highest amount at every retrogradation cycle. The dual-retrogradation of raw SDS was selected to produce SDS from sweet potato starch because the highest SDS content was obtained. Dual-retrogradation at 4°C for 48 hrs after hydrolyzed hydrolyzed by enzyme pullulanase was the best method that increased the SDS content of starch to 57.82%, higher than that of SPS. The triple-retrogradation led to a decrease in SDS content, suggesting unfavorable for modification of raw SDS to the production of SDS.

Conflict of interest

The authors declare no conflict of interest.

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