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In vitro Digestibility Study of Starch Complexed with Different Guest Molecules

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Digestibility of starch is an essential issue in food science studies due to its close relationship with human health. Most common starchy foods contain rapidly digestible starch, which can lead to chronic diseases, including type II diabetes. Heat–moisture treated potato starch (HPS) followed by inclusion complexation with guest molecules is prepared to improve starch's physicochemical properties, resulting in reduced digestibility. The guest molecules used in this study are linoleic acid (LA), stearic acid (SA), and sodium stearate (SS). The in vitro digestibility of the modified starches over time compared to native starch after gelatinization at 95 °C is examined. The starch complexed with SS results in the least amount of rapidly digestible starch (RDS), followed by LA and SA, consecutively. Furthermore, the starch-SS complexes are the most slowly digestible starch (SDS) and included the highest amount of resistant starch (RS), followed by LA and SA. Sodium stearate results in the highest transformation of RDS to be SDS and RS. Thermal analysis data and microscopy images support the digestion results.

1. Introduction

The digestibility of starch plays an essential role in human health. Glucose released from the starch metabolism is converted into energy and is used for human activity. However, its rapid digestibility rate can result in a high blood glucose level and lead to chronic diseases, including obesity, type II diabetes, and cardiovascular problems. Therefore, there is a growing interest in research on starch, that is slowly digestible or even partly undigested.[1–9]

Based on its resistance to enzyme digestion activity, starch is classified into three types, namely: rapidly digestible starch (RDS—digested in the small intestine to release glucose in 20 min), slowly digestible starch (SDS—digested in the small intestine to produce glucose in between 20 and 120 min), and resistant starch (RS—undigestible after 240 min but it is fermented in the large intestine by microorganisms to yield short-chain fatty acids).[10–15] Methods to assess the amount of reducing sugars and the molar mass distribution of amylopectin, amylose, and low molar mass sugars was developed by Ahmadi-Abhari et al.[16] Short digestion times (15 and 30 min) resulted in a reduction of oligomers and soluble sugars in the presence of lysophosphatidylcholine as a complexing agent, amylose is preserved after long digestion times due to the complexation.[16] The fermentation products yield (such as short-chain fatty acids, including propionate and butyrate) of resistant starch is greater than the yield from non-starch polysaccharide prebiotics such as pectin and probiotics such as yoghurt.[17] Hence, the research on slowly digestible and resistant starch products still attracts much attention.

In our previous studies, we investigated the effect of heat–moisture treatment (HMT) and inclusion complexation with different fatty acid types on the physicochemical properties of potato starch.[18–20] Linoleic acid (LA) as unsaturated, stearic acid (SA) as saturated, and sodium stearate (SS) as a salt form of a fatty acid were used as guest molecules, resulting in amylose complexes with different stability. The difference in complex stability due to various types of fatty acids as guest molecules may also result in different resistance to enzyme degradation. Hence, the digestion properties of those different complexes are reported in this article.

The effect of different fatty acid types on health aspects, such as cardiovascular diseases is reported in some literature.[21,22] Nettleton et al.[21] found that saturated fatty acids increased the amount of low-density lipoprotein (LDL) or ''bad'' cholesterol. A higher level of LDL in human blood increased the risk of heart disease problems. The authors also reported that the use of unsaturated fatty acids could reduce this risk. However, Zhu et al.[22] reported that both saturated and unsaturated fatty acids intake did not significantly affect the risk of cardiovascular disease. A high intake of trans-fatty acids had the most pronounced influence on cardiovascular disease. The current study focuses on in vitro digestibility of heat-moisture treated and complexed starch

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and does not further discuss the additional intake of fatty acids that comes with this method.

A cooking or baking process during food processing is inevitable. These processes affect the digestibility phenomenon as compared to unmodified starch. Raw or ungelatinized starch tends to be indigestible towards enzymatic digestion due to the protection of the tightly packed granules, resulting in a very small amount of sugars, whereas gelatinized starch results in a high amount of reducing sugars.[23,24] Our starch modification successfully improved the stability of amylose and increased the gelatinization temperature of starch, which is known to improve the resistance to enzymatic digestion.[18–20] Hence, in this study, we reported an in vitro digestibility analysis of heat-moisture treated and complexed starch, particularly after a gelatinization process at 95 °C. The enzymatic digestion process was conducted over time periods from 15 to 240 min. Complexation with linoleic acid, stearic acid, and sodium stearate resulted in different degradation profiles. HPS-SS complexes result in the highest reduction in reducing sugars amount, followed by HPS-LA and HPS-SA. Thermal analysis and microscopic observation were carried out to support the digestibility result.

2. Result and Discussion

A set of in vitro digestibility measurements was conducted to study the effect of the HMT followed by an inclusion complexation on the amount of reducing sugars released after various digestion times. Different types of guest molecules: linoleic acid, stearic acid, and sodium stearate, were investigated to provide information on the susceptibility of starch towards α-amylase. The experimental condition and the formulation of the samples for digestibility measurement are summarized in Table S1, Supporting Information. Thermal analysis and microscopy were used to confirm the digestion results. The effect of HMT and the complexation on the susceptibility of starch molecules by α-amylase is schematically illustrated in **Figure 1**.

2.1. Reducing Sugar Content after In vitro Digestion

Table S1, Supporting Information shows the experimental conditions used to prepare heat-moisture treated, complexed starches with various guest molecules; linoleic acid, stearic acid, and sodium stearate. The concentration of starch was 20% based on the total weight of the sample, while the concentration of guest molecules was weighed based on the dry matter of starch.

The amylase susceptibility on the gelatinized starches was determined by calculating the amount of reducing sugars released after a specific digestion time. The quantity of reducing sugars was spectrophotometric determined based on a DNSA method. Upon heating in the presence of reducing sugars, the 3-nitro (NO_2) group of DNSA reduces to an amino (NH_2) group, resulting in a color transformation of the DNSA solution from yellow to orange or red. The intensity of the color depends on the concentration of the reducing sugars present in the sample. The effect of both HMT and the starch-LA complexation after gelatinization on the in vitro digestibility can be observed in **Figure 2**. Native potato starch (without heat–moisture treatment and complexation) was used as a reference. Compared to NPS, there was no substantial effect on the reducing sugar reduction for HPS125, while HPS145 showed a slight decrease (Figure 2). However, the combination of heat–moisture treatment and complexation with guest molecules lowered the amount of reducing sugars and delayed the digestion process of the modified starch compared to the NPS. The reducing sugar content declined from 85% in NPS to 65% in the HPS145-LA complexes after gelatinization at 95 °C and a digestion time of 240 min (Figure 2). This result suggested that the main effect on lowering the digestibility of starch was due to the combination of HMT and inclusion complexes rather than HMT alone. HPS-LA was slower and less digested, which was referred to as 120–20 min digestion, and partly indigestible after 240 min. Figure 2 shows that HPS-LA required a longer time (240 min) to release the same amount of reducing sugars released by native starch after 120 min of digestion.

Figure 3 presents the amylase susceptibility on potato starch after HMT followed by stearic acid complexation and gelatinization at 95 °C in the RVA. The HPS-SA complexes prepared either in simulated tap water or in buffer reduced the amount of reducing sugars and delayed the digestion process of starch. The complexes prepared in buffer resulted in a lower amount of reducing sugars than in simulated tap water. Better solubilization of stearic acid in phosphate buffer facilitated the complex formation, leading to increased resistance towards enzyme digestion. Furthermore, the more pronounced effect was observed when the HPS145 rather than HPS125 was complexed with stearic acid (Figure 3). HPS145-SA resulted in a more significant decrease of reducing sugars than HPS125-SA (**Table 1**). In our previous study, we reported that the HPS125-SA and HPS145-SA largely reduced the swelling power.[18] Less swollen gelatinized starch granules due to the HMT and complexation decreased the susceptibility of starch molecules to α-amylase, hence, lowered the amount of reducing sugars.

The effect of the SS concentration during complexation on the amount of reducing sugars of HPS125-SS and HPS145-SS is presented in **Figure 4**. The amount of reducing sugar clearly decreased with an increase of the SS concentration. Even at 8% of SS, a decrease towards 5% was observed despite a concentration of 5% seems to be almost saturated.[20] After 120 min of digestion, the NPS released 69% of reducing sugars, while HPS125—2%, 5%, and 8% SS released 55%, 47%, and 40% of reducing sugars, respectively (Figure 4a). In the case of HPS145—2%, 5%, and 8% SS, the amount of reducing sugars released after 120 min of digestion were even lower, 53%, 43%, and 36%, respectively (Figure 4b). Furthermore, the rate of starch digestibility had been successfully slowed down. For example, in Figure 4a, to release 40% of reducing sugars, the HPS125—8% SS required a longer time (120 min) to be digested compared to the NPS, which was digested in less than 60 min. To release 55% of reducing sugar, the HPS125—8% SS required 240 min while NPS was digested only in 90 min. This result proved that the complexation with SS significantly slowed down the rate of starch digestion. The effect is even more pronounced in HPS145-SS complexes. In Figure 4b, to release 35% of reducing sugar, the HPS145—8% SS was digested slower after 120 min than NPS after 50 min. This outcome proved that the HMT followed by complexation with SS

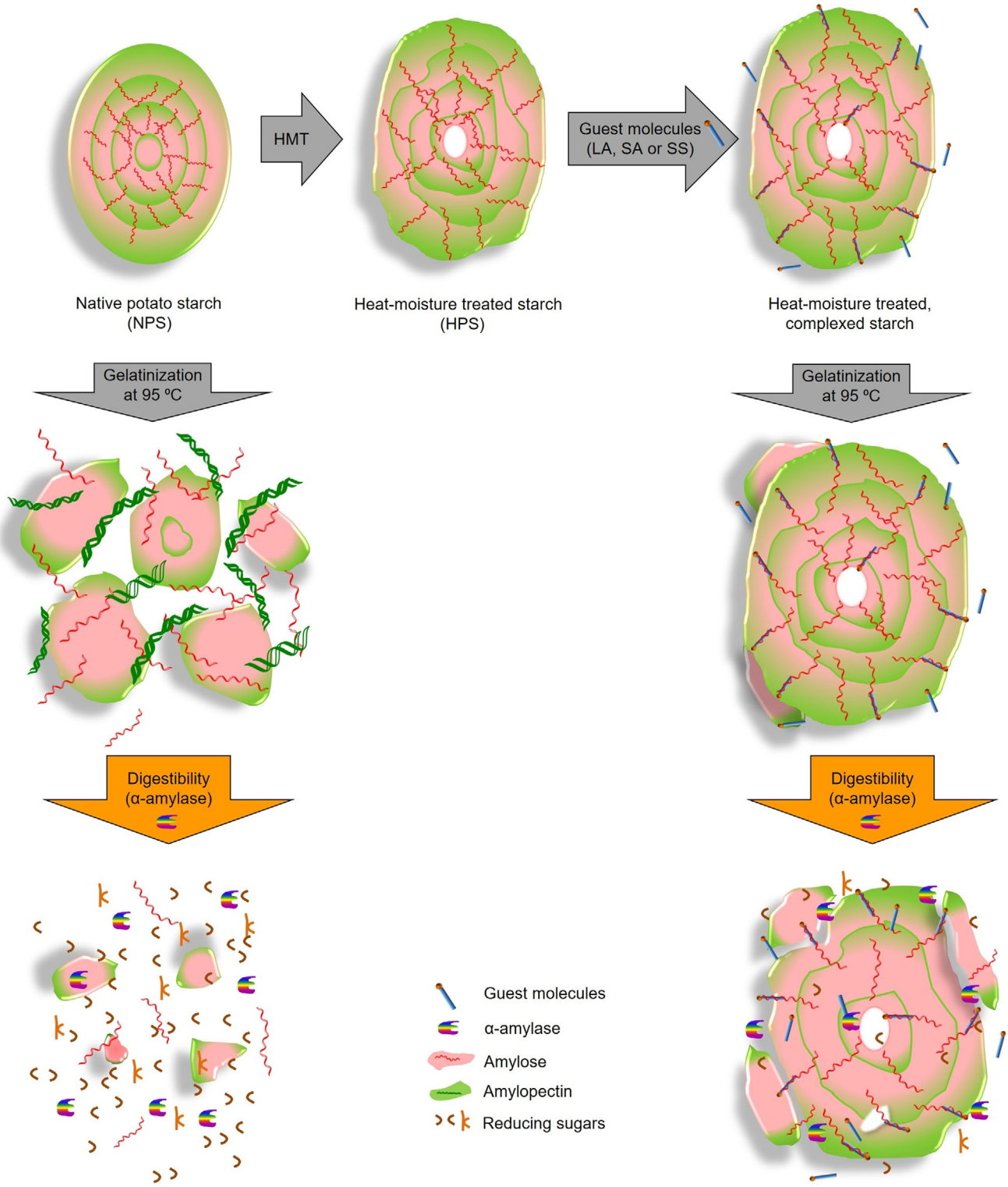

Figure 1. Illustration of the influence of HMT and complexation with guest molecules on the susceptibility of potato starch to α-amylase.

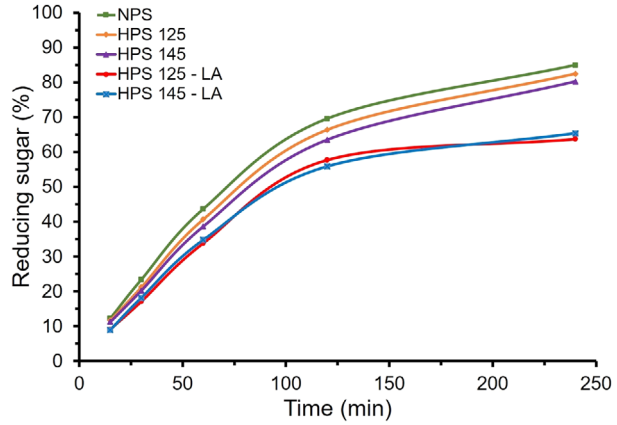

Figure 2. The influence of HMT and complexation with LA on the enzyme susceptibility of potato starch after gelatinization at 95 °C.

Table 1. Reduction in the amount of reducing sugar of heat-moisture treated starch-containing 5% linoleic acid, stearic acid, or sodium stearate in 10 dH compared to the reference (native starch), as a function of time.

Samples	Reduction in the amount of reducing sugar [%]				
	15 min	30 min	60 min	120 min	240 min
HPS 125—5% LA	26.0 (2.03)	27.0 (3.02)	22.6 (1.05)	17.1 (1.27)	25.0 (0.90)
HPS 145—5% LA	26.7 (2.19)	22.3 (4.30)	20.3 (2.48)	19.7 (1.91)	23.0 (1.53)
HPS 125—5% SA	10.7 (2.81)	17.1 (2.08)	16.1 (3.62)	21.5 (0.53)	22.7 (1.90)
HPS 125—5% Sa[a)]	12.9 (0.28)	17.5 (3.11)	17.3 (2.26)	20.6 (0.57)	21.2 (0.64)
HPS 145—5% SA	16.7 (7.22)	17.6 (1.40)	21.7 (0.92)	24.3 (1.27)	25.3 (2.96)
HPS 145—5% Sa[a)]	17.0 (0.28)	23.5 (1.06)	22.0 (2.97)	24.4 (1.77)	23.1 (0.99)
HPS 125—2% SS	46.8 (0.06)	30.1 (0.60)	28.2 (1.72)	20.5 (3.02)	16.3 (1.67)
HPS 125—5% SS	49.8 (1.78)	40.6 (6.28)	25.0 (5.08)	32.9 (2.40)	29.4 (0.12)
HPS 125—8% SS	59.9 (0.17)	55.8 (3.40)	54.7 (1.56)	43.7 (4.00)	35.1 (1.04)
HPS 145—2% SS	50.3 (2.98)	39.5 (2.15)	31.6 (0.89)	24.1 (4.37)	16.6 (0.17)
HPS 145—5% SS	58.5 (2.78)	43.2 (1.37)	40.6 (1.57)	38.6 (0.78)	33.2 (0.53)
HPS 145—8% SS	63.6 (0.52)	63.7 (2.43)	57.9 (3.86)	48.7 (1.04)	37.0 (1.11)

The values in the parentheses represent deviation standards ($n = 3$). [a)] Complexation was prepared in buffer.

resulted in significantly lower and slower digestible starch. The part that remained indigestible after 240 min of digestion is related to the amount of resistant starch, which was in the case of 8% SS with around 50% RS considerably higher than the approx. 40% RS for potato starch complexed with LA and SA. This resistant part was attributed to the stable complexes between amylose and guest molecules (**Figure 5**). Hence, less starch region could be digested by the enzyme, resulting in fewer reducing sugars (Figure 4).

The complexes between HPS-SS clearly showed the highest resistance towards enzyme degradation, demonstrated by the lowest amount of reducing sugars released and the highest reduction on the reducing sugars amount after digestion compared to HPS-LA and HPS-SA. The ratio of the amount of reducing sugars in the modified starch samples to the unmodified native potato starch was calculated as the "reduction of reducing sugars" (see Table 1). From Table 1 it is clear that the largest reduction in reducing sugars for each digestion time was obtained in the case of HPS complexed with SS. The reduction in the amount of reducing sugars in HPS-SS complexes was significantly ($p < 0.05$) higher than HPS-LA and HPS-SA complexes. This result can be explained by the larger amount and more stable complexes formed with SS compared with LA and SA (see Figure 5). The reduction in the amount of the reducing sugars at 30–240 min of digestion in HPS-LA complexes was not significantly different with HPS-SA complexes. The presence of amylose-guest complexes was considered as the resistant part of the starch.[16] The more complexes formed, the larger the crystalline portion of the obtained starch.[19] Due to the more stable orderly packed structure of the molecules in the starch complexes, the starch retained its ordered structure after the heating process. These structures were hardly attacked by the enzymes, hence lowering the enzyme susceptibility.

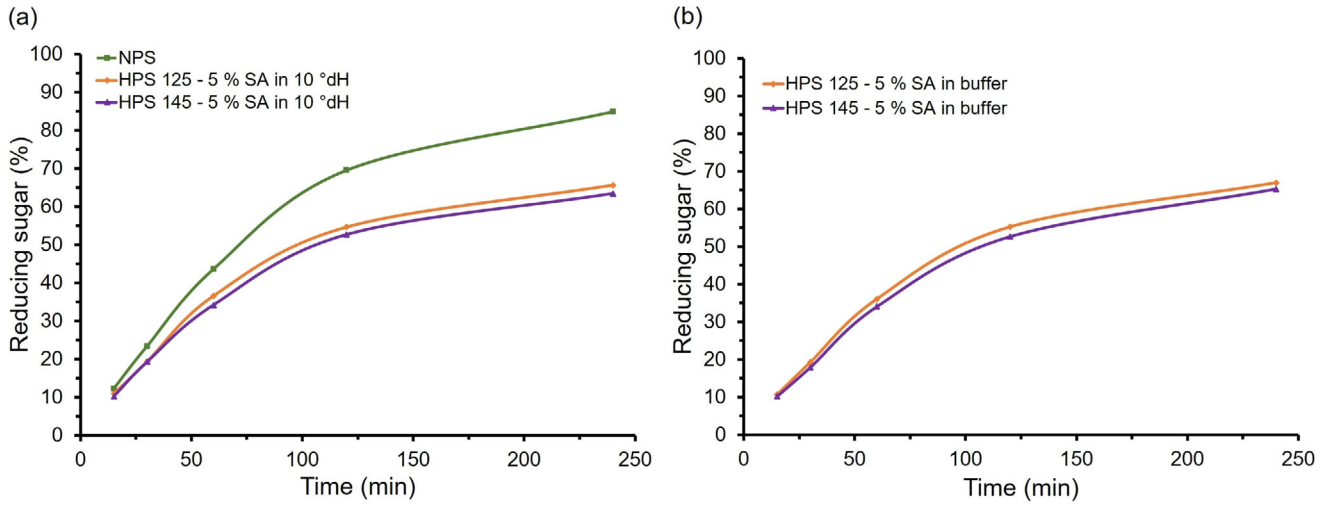

Figure 3. The influence of HMT and complexation with SA on the enzyme susceptibility of potato starch after gelatinization at 95 °C in a) 10°dH and b) phosphate buffer.

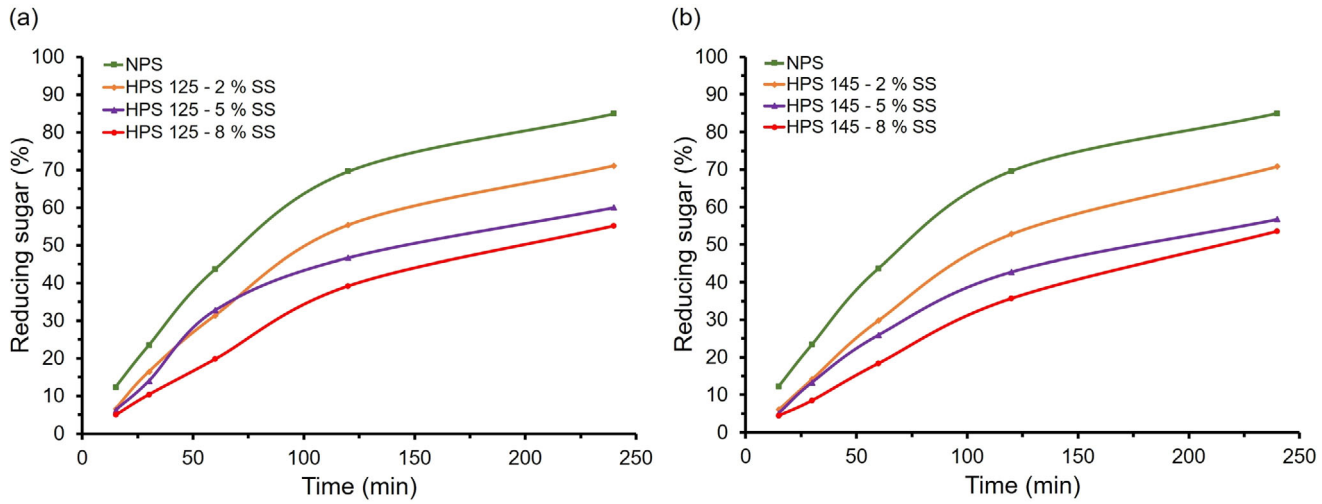

Figure 4. The influence of the SS concentration on the enzyme susceptibility of potato starch after gelatinization at 95 °C in a) HPS125—SS and b) HPS145—SS.

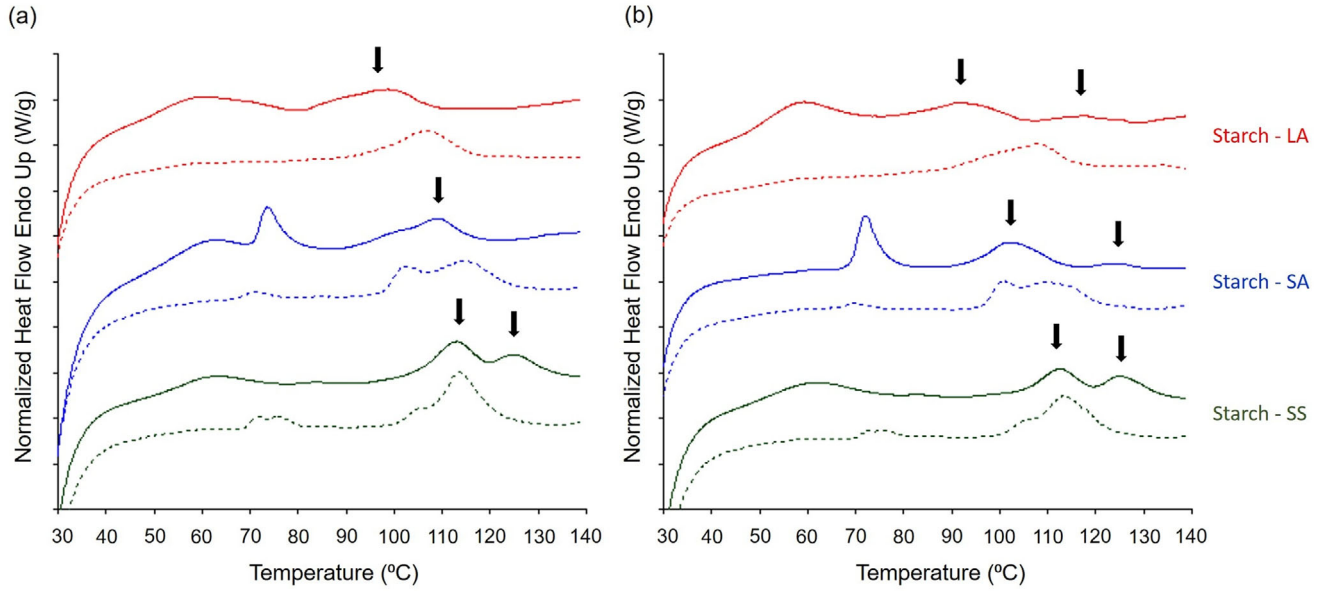

Figure 5. Thermal analysis of (20% w/w) of a) HPS125-complexed and b) HPS145-complexed with different guest molecules gelatinized at 95 °C, before (solid line) and after (dotted line) 240 min of digestion. The black arrow (→) is referred to the endotherm of the complexes.

2.2. Thermal Properties before and after Digestion

To detect that the amylose complexes were still present after a digestion time of 240 min, DSC was utilized to analyze the thermal properties before and after degradation, presented by respectively a red and blue DSC curve (Figure 5). All samples were initially gelatinized at 95 °C and then freeze-dried before DSC analysis. The endothermal transitions of the complexes remained observable after digestion, which indicated that the complexes were stable towards the enzyme degradation even after 240 min (see Figure 5). This result implied that the modified starch became less accessible for degradation by the enzyme due to the formation of the complexes.[10] However, in the case of LA and SS, the enthalpy of amylose-LA and amylose-SS complexes of the degraded starch samples were slightly lower than undegraded starch (see **Table 2**). The decreased enthalpy was caused by the loss of some less stable (less crystalline) complexes during digestion, and the most stable (well crystalline) complexes remained intact. In the case of stearic acid, the enthalpy of the complexes after degradation was higher than before (Figure 5). This phenomenon could be due to further complex formation during the heating process of the freeze-dried digested samples with the free stearic acid. However, the type II complexes clearly observed in the SS samples (before digestion) were disrupted after digestion and converted into type I complexes (Figure 5). The formation of amylose inclusion complex was referred

Table 2. Enthalpy changes (ΔH) of starch-complexes containing 5% linoleic acid, stearic acid, or sodium stearate before and after 240 min of digestion.

Sample	ΔH [J g^{-1}]	
	Before digestion	After digestion
HPS125—LA	4.0 (0.11)	3.8 (0.08)
HPS145—LA	4.7 (0.12)	4.0 (0.05)
HPS125—SA	4.4 (0.07)	7.4 (0.35)
HPS145—SA	4.8 (0.07)	7.3 (0.07)
HPS125—SS	8.8 (0.14)	7.5 (0.14)
HPS145—SS	9.0 (0.00)	7.3 (0.07)

The values in the parentheses represent deviation standards ($n = 3$).

to the resistant part of the starch which was enzymatically indigestible.[10,25]

Based on the thermal analysis curves in our previous study,[20] it was already expected that the HPS complexed with SS would be a promising candidate for slowly and resistant starch after cooking because the onset melting temperature (T_o) of the amylose-SS complexes was well above 100 °C. This outcome suggested that there will be not much increase on the amount of reducing sugar released when the starch samples are pre-gelatinized at 100 °C instead of 95 °C. In the case of amylose-SA complexes, boiling up to 100 °C can dissolve a small amount of the complexes, but the majority of the complexes will be retained, while in terms of amylose-LA complexes, the complexes will mainly melt (see Figure 5). This finding indicated that the amylose-SA complexes were thermally and mechanically more stable than amylose-LA complexes, resulted in more resistant physicochemical properties. Hence, we expected that the reducing sugars of HPS-SA would be lower than HPS-LA. However, in our findings, HPS-LA complexes resulted in a higher reduction of reducing sugars than HPS-SA complexes. A possible explanation is that in the case of linoleic acid, there was a better α-amylase protective film around the starch granules compared to stearic acid.[26]

2.3. Granular Structure after Digestion

Light microscopy was employed to observe whether the modified starch granules remained preserved after digestion compared to native starch. **Figure 6** clearly shows that NPS granules have been ruptured after gelatinization at 95 °C and disappeared after 30 min degradation. Longer degradation times caused the granules to break and split into more irregular forms. The granule structure of the NPS completely disappeared after 240 min of digestion. An HMT and complexation largely maintained the structure of the gelatinized starch granules, not only during the heating and shearing process but also after the enzymatic digestion, as shown in Figure 6. A large effect was observed on HPS-SS complex starch in which the whole granule structure mainly remained intact even after 240 min digestion. The regions preserved after a long digestion time were referred to the resistant part of the granules.[10] The HMT and complexation process reduced the digestibility of starch and increased the resistance towards enzyme digestion. Some starch structures retained after HMT and complexation proved that the enzyme hydrolysis during the digestion process was hindered. The microscopic results supported the in vitro digestibility results above.

3. Conclusion

Heat–moisture treatment followed by inclusion complexation successfully produced starches with improved stability towards heating and enzymatic digestion compared to native starch, which was confirmed by an in vitro digestibility analysis. During complexation, different guest molecules were used, including linoleic acid (LA), stearic acid (SA), and sodium stearate (SS). Based on the in vitro digestibility results, it can be concluded that the amount of RDS in the complexed starch follows the trend SA > LA > SS, while the highest amount of SDS and RS were obtained with SS, followed by LA and SA. This result proves that SS is the most promising guest molecule to form complexes, resulting in the most SDS and RS. Thermal analysis and granular morphology supported that the amylose complexes were largely stable towards the enzyme degradation. Further studies will investigate complexation with SS with other (HMT) starches and scale-up studies.

4. Experimental Section

Materials: Native potato starch with 13.4% moisture, 60–74% technical linoleic acid with a density of 0.902 g mL^{-1}, tween 80 with a density of 1.064 g mL^{-1}, span 80 with a density of 0.986 g mL^{-1} at 25 °C, stearic acid with reagent grade 95% (melting point 67–72 °C), casein sodium salt (CSS) from bovine milk, sodium stearate, or stearic acid sodium salt with purity ≥ 99% (GC), and the amount of sodium (Na) is 6.6–7.7%, Lugol (iodine/potassium iodide solution for microscopy), calcium chloride dihydrate (ACS reagent ≥ 99%, $CaCl_2 \cdot 2H_2O$), monosodium phosphate monohydrate, sodium phosphate dibasic, 3,5-dinitrosalicylic acid (DNSA), potassium sodium tartrate tetrahydrate, and maltose monohydrate were all purchased from Sigma-Aldrich Chemical Company. α-amylase from porcine pancreas (100 000 U g^{-1} on Ceralpha reagent at 40 °C and pH 6.9), as partially purified powder, was employed from Megazyme International Ireland (Wicklow, Ireland). Sodium chloride and sodium hydroxide were bought from Merck Company (Germany). All the chemicals were from analytical grade or better.

Preparation of Heat–Moisture Treated Potato Starch: The heat–moisture treated potato starch (HPS) was produced in a pressure vessel according to the previously reported method.[19] A pressure vessel was almost entirely filled with native potato starch and heated to 125 and 145 °C (the volume was approximately 34 cm^3) to obtain HPS125 and HPS145. After cooling to room temperature, the HMT starch was stored in a sealed container.

Sample Preparation: The samples were prepared in a Rapid Visco Analyzer (RVA-4) Newport Scientific (NSW, Australia). The HPS-LA complexes were prepared by mixing 9% starch on dm (w/w based on the total weight of a starch–water mixture of 28 g) in simulated tap water of 10°dH (0.2621 g L^{-1} of $CaCl_2 \cdot 2H_2O$ in distilled water) with an additional 3% (w/w based on dry matter of starch) of the combination of tween 80 and span 80 with a ratio of 1:3 respectively and 5% (w/w based on dry matter of starch) of linoleic acid. The linoleic acid was previously emulsified in water with tween 80 and span 80 in a homogenizer (Polytron PT 1300 D, Kinematica, Lucerne, Switzerland) at 10 000 rpm for 5 min. Emulsions for the HPS-SA complexes were prepared by mixing 5% of stearic acid and 10% of CSS (w/w based on dry matter of starch) in simulated tap water or phosphate buffer at 80 °C until well-dispersed stearic acid was obtained and then cooled to ambient temperature. For HPS-SS complexes, sodium stearate with various concentrations (2, 5, 8% w/w based on the weight of

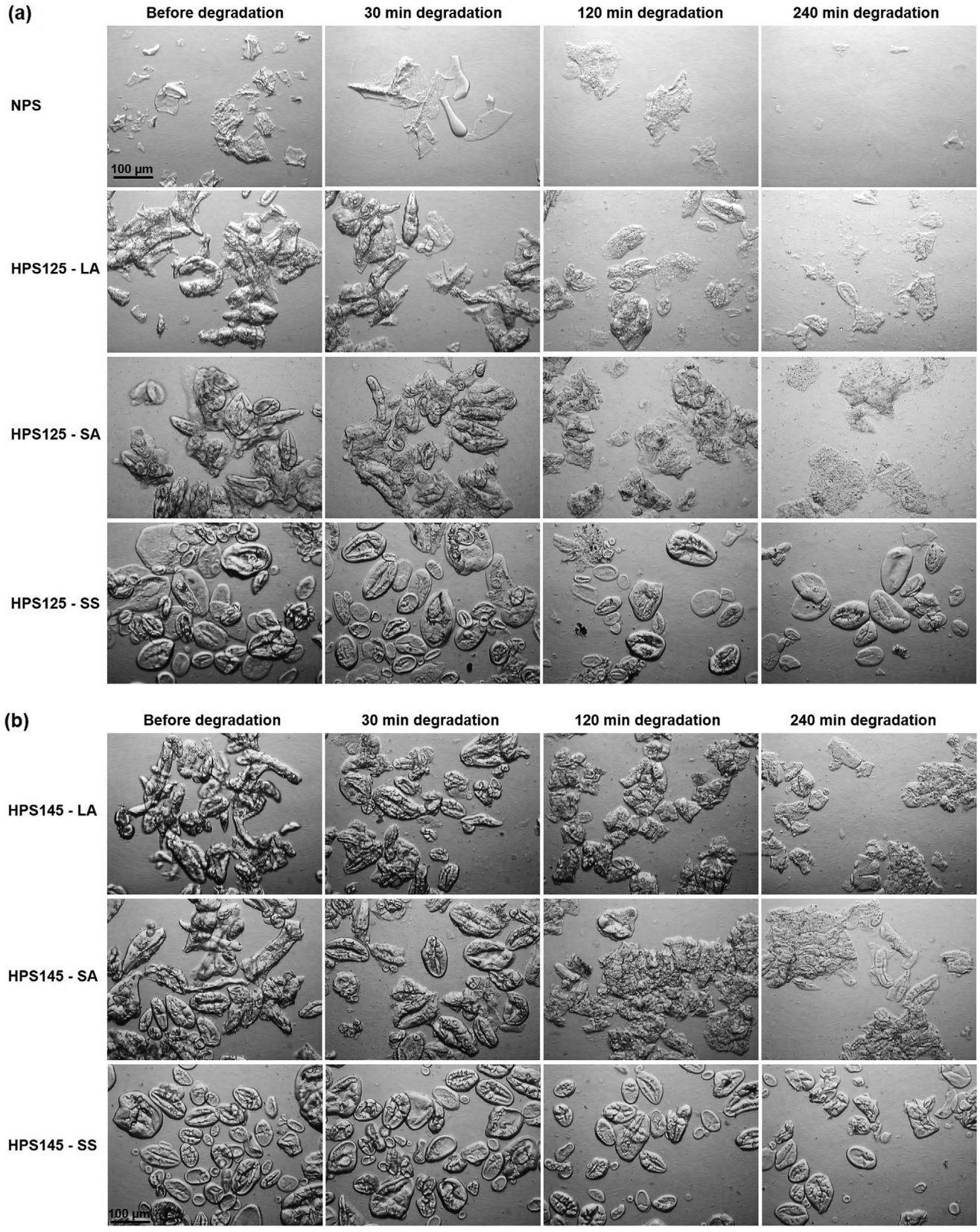

Figure 6. Light microscopy showing "granular" structures of a) HPS125 and b) HPS145 complexed with LA, SA, and SS before and after degradation for 30, 120, and 240 min.

starch) was solubilized in simulated tap water at 80 °C. Afterwards, a part of the suspension was transferred in an RVA cup, mixed with 9% (w/w) starch (total weight 28 g), and equilibrated for 10 min at room temperature before starting the RVA. Native starch with a concentration of 9% (w/w) in simulated tap water was used as a reference. The RVA profile was displayed as follows: equilibrated at 50 °C for 60 s, heated to 95 °C at a rate of 6 °C min^{-1} and held at 95 °C for 300 s. For the first 10 s, the rotation speed was 960 and 160 rpm for the rest of the profile. A part of the gelatinized starch samples were used for the digestibility study, and the rest was freeze-dried and stored for other analysis.

Preparation of DNSA Reagent: DNSA was weighed for 1 g and dissolved in 20 mL 2N sodium hydroxide and 50 mL 30% w/v of potassium sodium tartrate tetrahydrate solution. The mixture was gently stirred and heated to 72 °C until all solid was dissolved and a clear orange solution was achieved. The solution was cooled to the ambient temperature, and the volume was carried to 100 mL with distilled water. The DNSA reagent was stored in a dark container under N_2.

In vitro Enzymatic Digestion and Reducing Sugar Determination: The degradation study followed the previously reported method.[10] Maltose solution with five concentrations (2, 1, 0.4, 0.2, and 0.1 mg in 100 µL) were used as standards for the DNSA method to establish the calibration curve. All samples were measured in triplicate. An amount of 5 g of the hot cooked starch paste from the RVA was weighed (triplicate) into a 50 mL Greiner tube. Immediately, the hot paste was mixed with 17 mL phosphate buffer (17 g, 0.0025 M, containing 0.0075 M sodium chloride, pH 6.9) to obtain a 2% (w/v) starch suspension. The starch suspensions were shaken and homogenized using a homogenizer at 7000 rpm for 10 s, and then equilibrated for 5 min at 37 °C in a water bath to simulate a normal body temperature. A 0.05% porcine pancreas α-amylase solution was every day freshly prepared, of which 0.5 mL was added to each starch suspension. Subsequently, the starch suspensions were incubated at 37 °C while rotating in a modified ventilation oven (Thermo Scientific Heraeus 6000, Langenselbold, Germany). The degradation times were varied (15, 30, 60, 120, and 240 min). After each degradation time, the incubated solutions were pipetted 5 mL into different test tubes. The enzyme was immediately inactivated by heating the samples in a boiling water bath for 5 min. The samples in the test tubes were then cooled to room temperature. An amount of 100 µL of the degraded solutions was pipetted into a test tube, mixed with 2 mL distilled water and 1 mL DNSA reagent, and then vortexed. The rest of the samples were freeze-dried for further analysis in a laboratory freeze-dryer (Christ, Alpha 2–4 LD plus, Germany). Subsequently, the mixture in the test tubes was incubated for 5 min in a boiling water bath and then cooled to room temperature. The incubated solutions were diluted with 1 mL of distilled water. Next, the solutions were centrifuged in a Labofuge 400R at 1000 rpm for 15 min. Afterwards, the solutions were carefully brought into Eppendorf cups and then centrifuged in an Eppendorf centrifuge 5415 R at 10 000 rcf for 5 min. The absorbance of the supernatant of the samples was measured using a Hitachi U-1800 UV/vis Spectrophotometer (Japan) at 540 nm.

Thermal Analysis: A Perkin Elmer Pyris 1 Differential Scanning Calorimetry (DSC) was employed to analyze the thermal properties of the starch before and after degradation. The DSC was initially calibrated using indium (melting temperature = 156.6 °C and the enthalpy = 28.45 J g^{-1}). The freeze-dried samples were mixed with distilled water to a 20% (w/w) concentration and equilibrated for 1 h. Afterwards 55 µL of the starch suspensions was pipetted and weighed in stainless-steel pans from Perkin Elmer which were hermetically closed. The DSC scanning profile was; first heating–cooling–second heating (20–140 °C) at a rate of 10 °C min^{-1}.

Microscopy: A Nikon light microscope (Nikon, Eclipse 600, Japan) was utilized to observe the morphology of the gelatinized starch granules before and after degradation. Starch suspensions with a concentration of 1% in simulated tap water were initially prepared by dispersing the freeze-dried starch samples from the degradation study before and after each degradation time. The remained starch granules were observed under the bright-field illumination of the microscope. The magnification applied was 10×. A Nikon camera (Nikon, COOLPIX 4500, MDC Lens, Japan) was used for capturing the pictures.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

heat–moisture treatment, in vitro digestibility, starch inclusion complexes

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