



Dialdehyde Starch-Lysine Conjugates: Chemical, Structural and Functional Properties

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ABSTRACT

We produced a novel starch derivative in two steps: periodate oxidation to obtain dialdehyde starch (DAS) and conjugation of DAS with lysine in a Maillard-type reaction. Compared to the native tapioca starch, DAS has significantly lower intrinsic viscosity, molecular weight, relative crystallinity, smoothness of granule surface, and swelling power, but higher solubility and light transmittance. When produced using increasing periodate concentrations from 0.05 to 3.0 N, DAS had higher carbonyl contents and swelling powers but lower recovery efficiency, intrinsic viscosity, relative crystallinity, and solubility. When produced from DAS with higher carbonyl contents, the DAS-lysine conjugates had higher degree of lysine conjugation and swelling power but lower intrinsic viscosity, relative crystallinity, granule integrity and paste transparency. This approach of conjugation can be used in slow-release applications, where different amino acids, peptides, or even proteins can be conjugated with DAS, and then cleaved and released in the stomach or other acidic media.

Keywords: Dialdehyde starch, Amino acid conjugation, Periodate.

INTRODUCTION

In industrial applications, native starch exhibits many disadvantages in terms of technological properties. Therefore, food scientists and technologists have modified starches to change and improve their technological, physicochemical, chemical and digestive properties. There are three groups of starch modification techniques including chemical, physical and enzymatic treatments. At present, chemical modification is the most widely applied group of techniques. Chemical modification methods include oxidation, cross-linking,

esterification, etherification and cationization¹.

Many oxidizing agents have been used for starch oxidation, such as ClO_2^- , ClO^- , H_2O_2 , O_2^{2-} , IO_4^- , MnO_4^- , $\text{Cr}_2\text{O}_7^{2-}$, $\text{S}_2\text{O}_8^{2-}$ and O_3^{1-3} . In an acidic medium, periodate (IO_4^-) selectively oxidized and split the C-2 and C-3 bonds of the glucose units of starch with the formation of carbonyl (aldehyde) groups at those sites². In an alkaline medium, periodate oxidizes starch to form carboxyl groups with three mechanisms: (1) Cannizzaro reaction converting two aldehyde groups to a hydroxyl group and a carboxyl group, (2) a series of reactions begun



by β -elimination and then hemiacetal hydrolysis and a rearrangement of enzil-benzilic acid; (3) the hemiacetalization leading to the formation of internal cross-linkages⁴. After the periodate oxidation, the properties of the starch drastically change. Hemiacetalization helps to form internal crosslinks due to the presence of C=O groups. In addition, periodate oxidation also leads to depolymerization and production of carboxylic acids from dialdehyde starch (DAS). Dialdehyde derivatives of carbohydrates are known agents in crosslinking reactions with amino acids, proteins, pharmaceuticals, and leather⁴⁻⁶.

By attaching several new chemical functional groups to the starch molecule, starch derivatives acquire new chemical, structural and functional properties suitable for different applications. Lysine is an essential amino acid in proteins that supports the health of humans and animals. The carbonyl groups of DAS may conjugate with the amino group of lysine through a Maillard-type reaction, with the formation of an imine bridge between DAS and lysine. A few studies showed that hypochlorite-oxidized starch-amino acid conjugates have decreased solubility, swelling power, retrogradation, and enzyme digestibility, but to the best of our knowledge, there is no study on the properties of DAS-amino acid conjugates^{9,10}. Therefore, this work aims to produce and characterize the chemical, structural, and functional properties of DAS and DAS-lysine conjugates with varying degrees of periodate oxidation.

MATERIALS AND METHODS

Preparation of DAS

Sodium periodate (NaIO_4) was used to oxidize the tapioca starch (designated as S0) according to a reported method with slight modifications². Tapioca starch (200 g, dry basis) was added to 500 mL of NaIO_4 solution with different concentrations (0.05; 0.1; 0.2; 0.3 N). The reaction mixture was adjusted to pH 3.0 using 3 M HCl to begin the oxidation. After 1 h of continuous stirring at ambient temperature (30°C), the mixture was adjusted to pH 7.0 using 3 M NaOH to stop the reaction. Then, DAS was obtained by centrifugation at 3000xg for 15 min and washed 3 times with every 500 mL of deionized water. Next, the washed DAS was stirred with a $\text{Na}_2\text{S}_2\text{O}_5$ solution (500 mL,

0.5 %w/v) for 1 h to completely remove the excess periodate and iodate (NaIO_3). Then, the DAS was washed at least 3 times with deionized water and once with 70% ethanol solution using centrifugation at 3000xg for 15 minute. The DAS cake was dried in a convection oven at 45°C for 24 h, then ground and sieved (120 mesh). DAS samples (designated as S0.05, S0.1, S0.2, and S0.3 with the numbers presenting NaIO_4 normalities) were stored in zip lock bags under dry conditions at room temperature until further experiments.

Preparation of DAS-lysine conjugates

Conjugation of DAS with lysine was conducted according to a study described elsewhere¹⁰. DAS was suspended in DW at a 1:2.5 (w/v) ratio and then mixed with lysine to a 5% w/w ratio. The suspension was adjusted to pH 10 stirred at 40°C for 5 hours. The reaction was then terminated by adjusting the pH to 7.0. The solid was then obtained by centrifugation (3000xg, 15 min) and washed at least 4 times with DW and finally with 70% ethanol. To confirm the absence of lysine residue, the supernatant (1.0 mL) was added to a test tube with 8 drops of 20 mg/mL ninhydrin solution in ethanol and the mixture was boiled for 10 minute. The absence of color change in the solution indicated that there was no lysine residue left in the supernatant. The DAS-lysine conjugates were then dried in a convection oven at 45°C for 24 h, then ground, sieved (120 mesh) and stored in zip lock bags at room temperature for further experiments. The conjugates were designated as C0.05, C0.1, C0.2, and C0.3 with the numbers indicating NaIO_4 normalities used when preparing the DAS.

Carboxyl (-COOH) and carbonyl (-CHO) contents in DAS

The carbonyl content in DAS was determined following a published procedure¹¹. A suspension containing 2.0 g dry starch and 50 mL of DW was boiled for 20 min with continuous stirring to completely gelatinize the starch and then cooled to room temperature. Then, a hydroxylamine reagent (30 mL) was added and the mixture was stirred in a water bath (40°C, 4 hours). At the end of the reaction, the excess hydroxylamine was determined by titrating the mixture to pH 3.2 with 0.1 N HCl solution. Blank determinations were performed similarly without starch samples. The hydroxylamine reagent was prepared by mixing 25 g of $\text{NH}_2\text{OH}\cdot\text{HCl}$

in 100 mL of 0.5 N NaOH solution and making up to 500 mL with distilled water.

The carbonyl content was estimated as follows:

$$\text{Carbonyl content (\%)} = \frac{0.028 \times N \times (V_B - V_S) \times 100}{W} \quad (1)$$

Where $N=0.1$ mol/L was the molarity of HCl; V_B and V_S were the volumes (mL) of HCl solutions used to titrate the blank and the DAS, respectively; and $W=2.0$ g was the mass of DAS.

The carboxyl content of DAS was determined using another procedure¹². The DAS (2.0 g, dry basis) was mixed with 25 mL of 0.1 N HCl solution and stirred for 30 minute. The DAS was then centrifuged and washed with DW 4 times, and the final supernatant was tested for the absence of chloride ions using a 0.1 N AgNO₃ solution. Distilled water (300 mL) was added to the starch cake and the mixture was boiled (20 min, continuous stirring) for complete gelatinization. Then, the starch solution was titrated with 0.01 N NaOH using phenolphthalein as the pH indicator. Blank experiments were done with the native starch.

The carboxyl content in DAS was calculated using the equation:

$$\text{Carboxyl content (\%)} = \frac{0.045 \times N \times (V_s - V_b) \times 100}{W} \quad (2)$$

where $N=0.01$ mol/L was the molarity of NaOH; V_s and V_b were the volumes (mL) of NaOH solution used to titrate the DAS and the blank, respectively; and W was the dry weight (g) of the DAS.

Total nitrogen content

The lysine content in DAS-lysine conjugate was expressed as total nitrogen content (N%), which was determined using the Kjeldahl method¹³.

Viscosities and Average Molecular Weight (M_w)

Intrinsic viscosity (η_i , mL/g) correlates to M_w of starch molecules in alkaline solutions. Starch solutions (from 1.0 to 5.0 mg/mL) were prepared by dissolving in 1 M KOH. A viscometer (Ostwald, $\eta=0.3$ mm, Ref. No 509 03, Germany) was used for measuring kinematic viscosity (η , m²/s) at 30-70°C, which was used to calculate other quantities, including the relative viscosity (η_{rel})= η/η_o (η, η_o are kinematic viscosities of starch and 1 M KOH

solutions, respectively), the reduced viscosity (η_{red})= $(\eta_{rel}-1)/c$ (c is the concentration of starch in potassium hydroxide solution), and intrinsic viscosity (η_i)= $\lim_{c \rightarrow 0} \eta_{red}$. Then, M_w of starch was estimated as $\eta_i=KM^\alpha$ ($K=1.18 \times 10^{-3}$; $\alpha=0.89$)¹⁴⁻¹⁶.

Fourier-Transform Infrared (FTIR) Spectroscopy

FTIR spectra were obtained at the wavenumbers from 400 to 4000 cm⁻¹ using an FT/IR-4700 spectrometer (Jasco, Japan) with ATR PRO ONE single-reflection ATR accessory. The ratio of alpha-helix to amorphous (RAA) was calculated as the relative absorbance intensity of 1047/1022 cm⁻¹ peaks (alpha helix region/amorphous region).

X-ray diffraction measurement

X-ray diffraction (XRD) patterns of samples were recorded using a powder X-ray diffractometer (Model D5005, Bruker, Germany). The conditions of the operation were 40kV and 40mA with Cu-K α radiation of 0.15406nm (Nickel filter; time constant=4 s). Each scan was done with 2theta from 3 to 30°. The degree of relative crystallinity (DRC) was estimated using the Origin software (version 7.5, OriginLab, USA):

$$\text{DRC} = \frac{A_c}{A_c + A_a} \quad (3)$$

Where A_c and A_a are the areas of crystalline and amorphous regions¹⁷.

Morphological characteristics

The morphological properties of starch granules were taken using an SEM-S 4800 field-emission scanning electron microscope (Hitachi, Japan) at 75 kV.

Functional properties

Swelling power (SP) and solubility index (SI) were determined using a modified procedure described elsewhere¹⁸. DAS ($m_1=1.0$ g) was dispersed in DW (30 mL) in a 50-mL falcon tube and then mixed carefully. The suspension was incubated in a water bath (30-95°C, 30 min), then cooled to ambient temperature and centrifuged (1000 \times g, 15 min) to obtain a wet cake (m_2) and a supernatant. The supernatant was dried at 105°C for 24 h in a convection oven to a dry solid (m_3). SI and SP were calculated using the following formulae:

$$\text{SI (\%)} = \frac{m_3}{m_1} \times 100 \quad (4) \quad \text{and} \quad \text{SP} = \frac{m_2}{m_1 - m_3} \quad (5)$$

Light transmittance (LT) of starch paste was estimated using a published method¹⁹. Starch suspensions (0.5 to 4%w/v) were boiled for 30 min for complete gelatinization and then cooled to room temperature. LT(%) of the starch pastes were measured at 660nm using a UH-5300 UV-Vis spectrometer (Hitachi, Japan) with distilled water as the blank.

Statistical analysis

All experiments were triplicated and reported in mean values. All standard deviations were lower than 10% of their means and were not shown. Analysis of variance (ANOVA) and Duncan's test for differences between means ($p < 0.05$) were conducted using SPSS software (ver. 17.0, SPSS, USA).

RESULTS AND DISCUSSION

Carboxyl, carbonyl and total nitrogen contents

The periodate oxidation is known to cleave the C2-C3 bonds in the anhydroglucose units of starch to form two carbonyl groups⁵. These carbonyl groups are mainly aldehydes since the oxidative cleavage mainly involves secondary hydroxyl groups in the starch structure²⁰. Besides, some -OH groups of some α -D-glucose units can be randomly oxidized to carboxyl groups due to the disruption of the glucose rings and the depolymerization⁶. Fig. 1 shows a high selectivity of periodate oxidation in producing carbonyl groups, which is beneficial in the formation of DAS-aminoacid conjugates due to their high affinity toward amino groups.

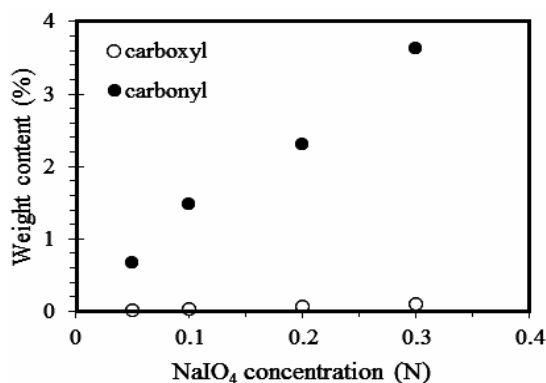


Fig. 1. Carbonyl and carboxyl contents of DAS as affected by NaIO₄ concentration

Figure 2 shows that the total nitrogen contents in the DAS-lysine conjugates were significantly different from those in DAS, indicating

the presence of lysine moieties in the conjugates. Fig. 2 and Fig. 2 indicated that the nitrogen contents in the DAS-lysine conjugates were positively correlated with the carbonyl content in DAS, confirming that the conjugation was due to the Maillard-type reaction between the -CHO group in DAS and the -NH₂ groups in lysine⁹.

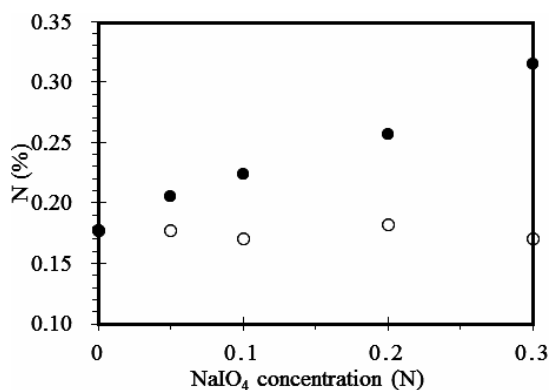


Fig. 2. Total nitrogen content in DAS (o) and DAS-lysine conjugates (•)

Intrinsic viscosity and average molecular weight

Table 1 shows that the molecular weight of DAS and intrinsic viscosity of their gelatinized solutions decreased with higher periodate concentrations during the oxidation step, possibly due to increased depolymerization^{2,21}. Kawaljit Singh Sandhu *et al.*, (2008) suggested that oxidative degradation is more susceptible to linear amylose molecules than to amylopectin²². Besides, the intrinsic viscosity of DAS-lysine conjugates samples increased slightly compared to that of DAS but was still much lower than that of native starch. This might be due to lysine complexing with starch causing an increase in molecular weight, increasing the bulkiness of the starch molecule, thereby increasing the intrinsic viscosity.

FTIR spectra

The absorbance bands in the FTIR spectra of DAS and DAS-lysine conjugates (Fig. 3) agreed with many previous studies. The bands at 1157 and 1075 cm⁻¹ belonged to the coupling of C-O, C-C stretching, C-O-H bending. The peak at 995 cm⁻¹ was caused by skeletal mode vibrations of the C-O-C glycosidic bridge²³. The bands at 3300 and 2920 cm⁻¹ were due to the O-H and C-H bond stretching, respectively³. The region 2380-2460 cm⁻¹ is due to the presence of CO₂ in the air and hence is not discussed. The peak at 1628 cm⁻¹ was characteristic of the tightly bound water in the starch²⁴.

Table 1: Intrinsic viscosity (η_i) and average molecular weight (M_w) of native starch (S0), DAS (S series) and DAS-lysine conjugates (C series)

| Sample | η_i | | | | | M_w (105 g/mol) |
|--------|---------------------|---------------------|---------------------|---------------------|---------------------|----------------------|
| | 30°C | 40°C | 50°C | 60°C | 70°C | |
| 0 | 273.35 ⁱ | 219.95 ^h | 178.65 ^g | 141.69 ^j | 130.77 ⁱ | 5.95 ^a |
| S0.05 | 22.04 ^g | 17.93 ^f | 16.43 ^e | 10.12 ^d | 9.56 ^d | 0.63 ^d |
| S0.1 | 12.12 ^e | 10.19 ^c | 9.62 ^d | 9.01 ^e | 7.95 ^e | 0.37 ^c |
| S0.2 | 10.07 ^c | 9.16 ^b | 8.16 ^c | 7.14 ^c | 6.40 ^c | 0.32 ^b |
| S0.3 | 8.47 ^a | 7.80 ^a | 6.02 ^a | 5.56 ^a | 3.39 ^a | 0.27 ^a |
| C0.05 | 24.77 ^h | 19.63 ^g | 19.13 ^f | 17.01 ^h | 13.62 ^h | NA |
| C0.1 | 14.24 ^f | 12.17 ^e | 10.07 ^d | 9.89 ^f | 8.80 ^f | NA |
| C0.2 | 11.26 ^d | 10.54 ^d | 9.47 ^d | 8.02 ^d | 6.96 ^d | NA |
| C0.3 | 9.64 ^b | 7.80 ^a | 7.28 ^b | 5.97 ^b | 4.95 ^b | NA |

Numbers in a column with different superscript letters are significantly different ($p < 0.05$). ND: not available, because no factor to calculate M_w from intrinsic viscosity was found for DAS-lysine conjugates.

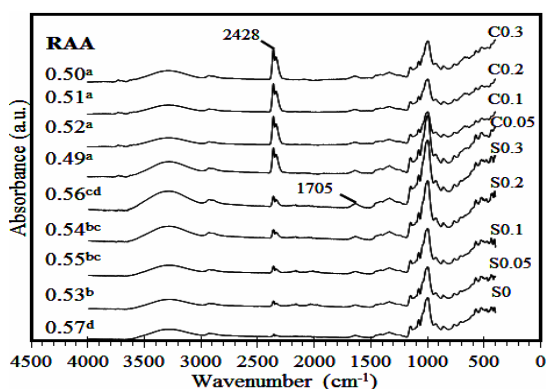


Fig. 3. FTIR spectra and ratio of alpha-helix to amorphous (RAA) of samples. RAA values with different superscript letters are significantly different ($p < 0.05$)

Figure 3 shows that the intensity of the carbonyl (C=O) stretching vibration in the region of 1781-1690 cm^{-1} increases proportionally to the carbonyl content in the DAS samples (Fig. 2)^{3,25}. Besides, in the Maillard reaction, the C=O group of DAS reacted with the amine (R-NH_2) group of lysine to produce the imine group C=NHR . Thus, after the Maillard reaction, the number of C=O groups decreased, leading to the reduction in the intensity of the peak 1705 cm^{-1} .

The ratio of intensities of 1047/1022 cm^{-1} peaks exhibited the degree of order in starch¹⁶. The RAA (ratio of α -helix to amorphous structures) of samples were ordered as raw starch > DAS > DAS-lysine conjugates. This can be explained by the partial starch hydrolysis occurring in the α -helix structure region during the periodate oxidation in a slightly acidic solution. However, the RAA in the DAS gradually increased in proportion to

the periodate concentration. Thus, the presence of crosslinks based on (hemi-)acetal formations possibly increased the RAA in these samples⁵.

The DAS-lysine conjugates were not significantly different in RAA values ($p > 0.05$). With the same oxidation degree, DAS had a lower RAA than that of the DAS-lysine conjugate. This can be explained by the fact that lysine was conjugated with the starch molecule, making the starch structure bulky, hence reducing the intermolecular association and thereby the RAA²⁶.

X-ray diffraction (XRD) and degree of relative crystallinity (DRC)

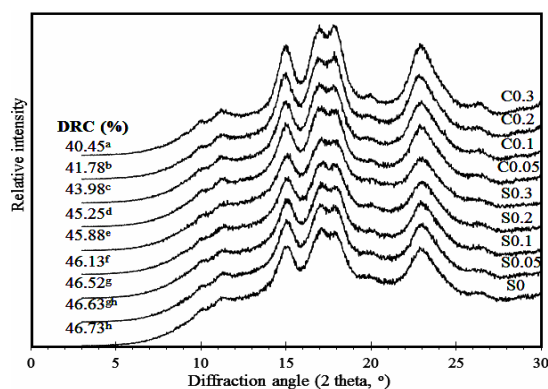


Fig. 4. X-ray diffraction spectra and degree of relative crystallinity (DRC) of samples. DRC values with different superscript letters are significantly different ($p < 0.05$)

Figure 4 showed that 2θ peaks typical for A-type starch crystals were present at 15.4, 17.5, 18.3, and 23.4° on XRD patterns of native starch, DAS and DAS-lysine conjugates, respectively^{16,27}. Thus, neither oxidation nor lysine conjugation

changed the A-type crystal of tapioca starch. DRC of DAS samples gradually decreased with higher degrees of periodate oxidation. The oxidation reaction occurred mainly in the amorphous and semi-crystalline regions of starch, resulting in a decrease in DRC³. Next, in the Maillard reaction, lysine was conjugated with starch molecules making the starch bulkier, or in other words, changing the structure of starch, thus reducing the association in DAS-lysine conjugate and further reducing DRC²⁶.

Morphological properties

The surface of native starch granules was oval to round shape with a smooth surface, and many granules had a large indentation on the surface and were relatively free from imperfections (Fig. 6). Previous studies have shown that oxidation can create large holes on the surface of starch granules¹⁶. However, Kawaljit Singh Sandhu *et al.*, (2008) found insignificant differences in the appearance of the native and the oxidized starch granules. The authors stated that the degree of oxidation was not sufficient to cause any remarkable change in the morphology

of starch granules²². This insignificant change can be observed in the SEM micrographs of S0, S0.05, S0.1, and S0.2 samples. Alternatively, oxidation can crack starch granules into smaller particles due to the starch crystalline structure weakening. This can be seen clearly when comparing the particle sizes, especially between S0 and S0.3 samples.

Jihong Li *et al.*, (2010) showed that during conjugate with lysine, hypochlorite-oxidized potato starch granules were eroded and roughened¹⁰. The more the number of carbonyl groups was, the more derivatization of DAS with lysine (from which the more starch granules were broken down) was. The results in Fig. 4, 5 and Table 1 agreed that the periodate oxidation and the lysine conjugation occurred mostly in the amorphous structure and, to some extent, in the semicrystalline one, resulting in the cracking of starch at those amorphous and semicrystalline sites and leaving the crystalline rings (fragments of layers) observed in the SEM micrographs, especially for the C0.2 and C0.3 samples²⁸.

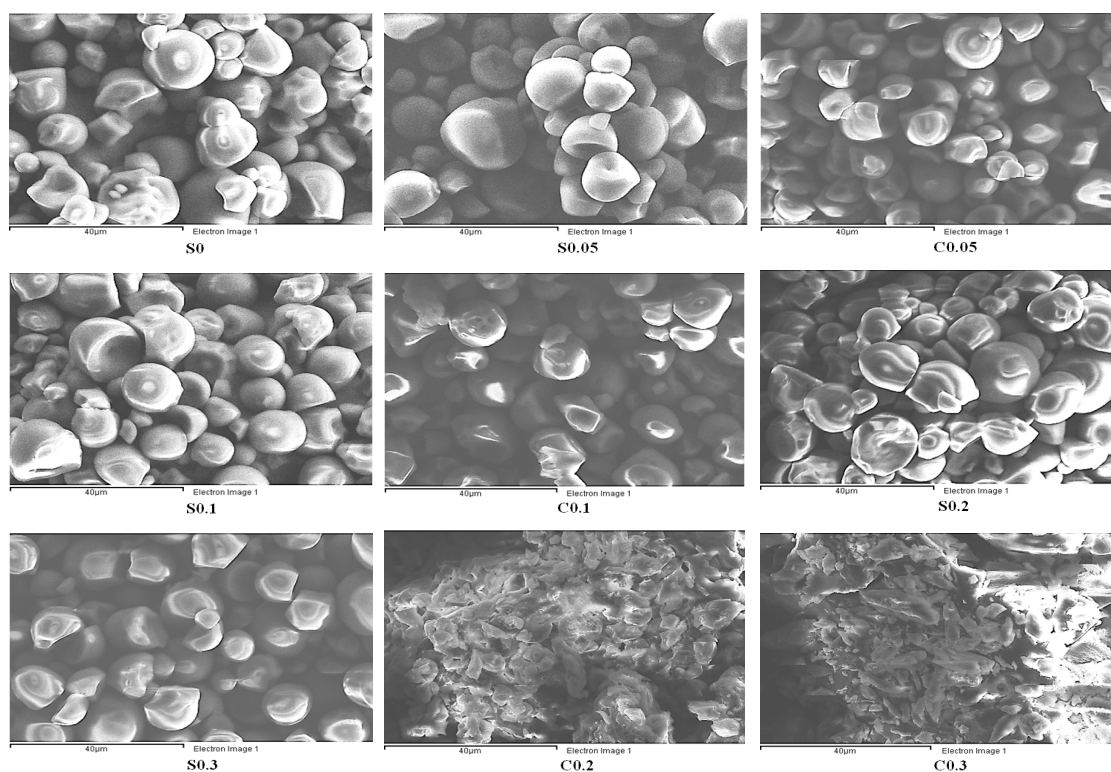


Fig. 5. SEM micrographs of starch, DAS, and DAS-lysine conjugates powders

Swelling power (SP)

When starch is suspended in water at low

temperatures, the starch granules absorb water and swell. Fig. 6A showed that in the temperature

range of 30-60°C, there was no significant difference in SP of DAS samples. Differences in SP became clearer at temperatures higher than 60°C. SP of DAS samples at 95°C ranked in the following order: S0.05<S0.1<S0.2<S0.3<S0. This result shows that periodate oxidation resulted in lower SP of DAS compared to that of the native starch. The reduction in SP after oxidation was reported for Mucuna bean starch, normal and waxy corn starches, and rice starch²⁹.

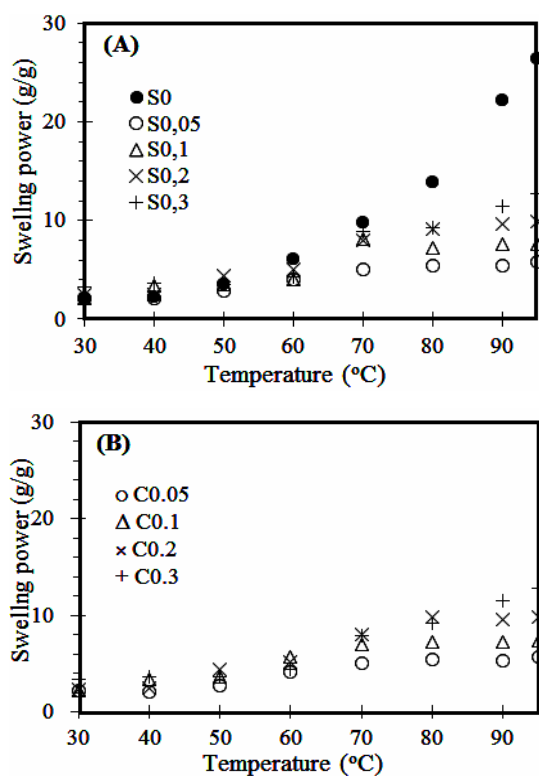


Fig. 6. SEM micrographs of starch, DAS, and DAS-lysine conjugates powders

SP represents the water capacity of starch under specific temperature conditions. Depending on the origin of native starch and the nature and degree of modification, their swelling and water solubility are different. Binding forces between particles also affect SP. The micellar structures of tightly bound starch granules are more repellent of swelling, while the amorphous domain is more easily affected by hydration and consequent swelling³⁰. S. Veelaert *et al.*, (1994) stated that at low to moderate periodate oxidation levels (10-50%), the crystalline region of starch partially converts to amorphous (due to hydrolysis of starch molecules) which increases the hydrophilicity of the granules resulting in increased

SP5. However, when the oxidation level exceeds 60%, another phenomenon prevails. The presence of carbonyl groups, whether they are involved in (hemi-) acetals or not, causes the starch molecules to become less water-affinity (more hydrophobic) thereby reducing SP. Also, it is possible that the presence of crosslinks based on (hemi-)acetal formation also reduced SP. Thus, DAS in this study was oxidized to a fairly high degree leading to the depolymerization of starch molecules, and a rise in the amorphous region (Fig. 4-5 and Table 1). However, the presence of a large amount of carbonyl hydrophobic group (Fig. 2) was the main reason for low SP.

Figure 7A and 7B also show that there is no significant difference in SP of each DAS with its lysine conjugate. A previous study also reported that potato starch-lysine conjugate also had SP similar to that of the original starch⁹. This phenomenon is interesting because the amino acid moiety is hydrophilic and should increase SP after replacing a $-\text{CH}=\text{O}$ group in DAS. We suggest that the lysine moiety could form crosslinks with each other and/or with the carboxyl groups in DAS, thus suppressing the swelling of DAS.

Solubility index (SI)

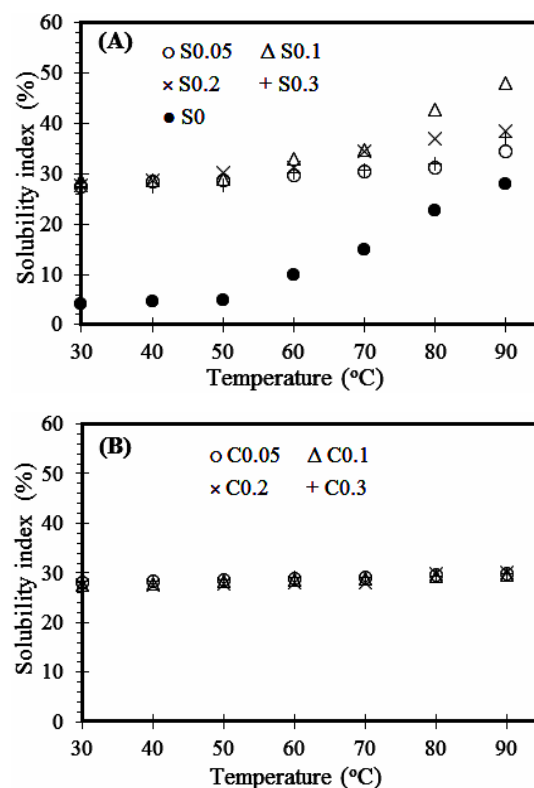


Fig. 7. Solubility index of (A) DAS and (B) DAS-lysine conjugates

Solubility index (SI) is the portion of starch granules that escaped after swelling at a specified temperature. The SI of native starch was lower than that of DAS at temperatures of 30-95°C (Fig. 7A). From 30-60°C, the SI was almost unchanged. However, at temperatures higher than 60°C, SI increases with temperature due to the escape of some molecules from the starch granules to water². When incubated at 95°C, SI of the DAS samples were $S0 < S0.05 < S0.3 < S0.2 < S0.1$ (Fig. 7A). Thus, at low concentrations of NaIO₄ (0.1 N), starch molecules were depolymerized to form low-molecular-weight fractions and were easily soluble in water, thereby increasing SI^{2,21}. In contrast, at higher periodate concentrations, a certain amount of formed cross-linkages reduced the number of starch molecules released after swelling, thereby reducing SI. But most importantly, the presence of many hydrophobic aldehyde groups instead of hydroxyl groups was the main cause of the decrease in the solubility of DAS⁵.

It was reported that starch-poly(lysine) conjugates reduced SI, while starch-lysine conjugates had almost the same SI, compared to that of the native starch⁹. In our study, all DAS-lysine conjugates had almost the same SI (Fig. 7B). Interestingly, the SI of all DAS-lysine conjugates remained almost unchanged in the whole temperature range of 30-95°C. In other words, even a low content of lysine attached to DAS could significantly suppress the solubility of DAS even at high temperatures. Moreover, the SI of DAS-lysine conjugates tended to be lower than that of their DAS counterparts (Fig. 7A and 7B). These phenomena can be explained by the lysine, which was conjugated to starch molecules, inhibited the emigration of molecules from the starch granules into water⁹.

Light transmittance (LT)

Figure 8A showed that at low concentrations ($\leq 2\%$), LT of DAS pastes was ordered as $S0 < S0.05 \approx S0.1 < S0.2 \approx S0.3$. However, at higher concentrations, LT of DAS pastes was similar to each other and was higher than LT of the native starch pastes. The increase in LT after periodate oxidation is due to the -OH groups being displaced by mostly -CHO groups (and a small amount of -COOH). Kawaljit Singh Sandhu *et al.*, (2008) stated that adjacent starch molecules were pushed apart, thereby reducing interchain association and resulting in increased LT²². Under gelatinization, starch granules

were swollen, starch molecules were dissociated and light can penetrate the paste more easily.

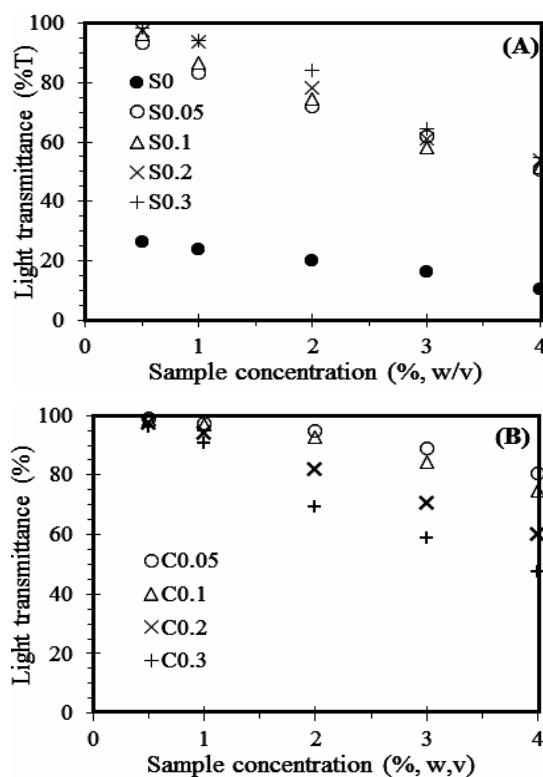


Fig. 8. Light transmittance (LT) of DAS (A) and DAS-lysine conjugates (B) solutions

Furthermore, DAS-lysine conjugate pastes had higher LT than their DAS counterparts. Lysine conjugated with starch molecules reduced the association in the conjugates thereby increasing their LT²⁶. However, LT of the conjugates (especially at 95°C and at high sample concentrations) is ranked in the order $C0.3 < C0.2 < C0.1 < C0.05$. This means that a higher degree of conjugation decreased the paste LT. This can be explained by the fact that a certain amount of amino acids conjugated with starch molecules makes the structure of starch bulkier and reduces the mobility of the starch chains, thereby reducing the LT^{9,26}.

CONCLUSION

In this study, we synthesized DAS and its conjugate with lysine as a model essential amino acid. The chemical and structural characterization techniques confirmed the formation of DAS and DAS-lysine conjugate, and several functional properties of them were investigated. This type of conjugate

can be used in slow-release applications, where different amino acids, peptides, or even proteins can be conjugated with DAS, and then cleaved and released in the stomach under acidic conditions. However, many further studies are still required for this approach to find real-world applications.

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Conflict of interests

All authors declare that they have no conflicts of interest to disclose.

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