DE GRUYTER e-Polymers 2023; 23: 20228090

Research Article

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Effects of dry heating, acetylation, and acid pre-treatments on modification of potato starch with octenyl succinic anhydride (OSA)

https://doi.org/10.1515/epoly-2022-8090
received October 13, 2022; accepted November 29, 2022

Abstract: Octenyl succinic anhydride (OSA) starch is widely used to stabilize emulsions. Nevertheless, the poor compatibility of starch with hydrophobic groups has restricted the performance of OSA modification. In this work, potato starch was pre-treated once or twice (dry heating, acetylation, and acid modification) prior to OSA modification. Pre-treatments increased the degree of substitution (DS), hydrophobicity, hydrophilicity, and decreased amylose content of OSA starches, with dual pre-treatments having greater effects. Among all pre-treatments, acid modification followed by dry heating resulted in the greatest OSA modification (DS: 0.015) and water-binding capacity (155%). Meanwhile, acid modification followed by acetylation produced OSA starch with the highest oil-binding capacity (290%). Scanning electron microscopy revealed that the granular deformation of dual pre-treated OSA starches was greater compared to single pre-treated and non-pre-treated OSA starches (O). Dual pre-treated OSA starches (ADO, 7%; ACO, 8%) had lower amylose contents than those of single pre-treated (AO: 12%, CO: 17%, DO: 21%) and O (36%). All the pre-treatments reduced the setback viscosity of OSA starch to a lower range (70–394 cP), simultaneously decreasing their retrograde tendency. This study suggested that dual pre-treatments could improve the efficiency of OSA modification and produce OSA starch with greater emulsifying potential.

Keywords: acetylation, acid modification, dry heating, OSA modification, potato starch

1 Introduction

Starch has been widely employed in food, pharmaceutical, and industrial applications around the world because of its abundance, low cost, renewable nature, biocompatibility, biodegradability, and non-toxic property. Due to the growing demands for environmental, natural, and label-friendly ingredients, starch has garnered considerable attention as an emulsifying agent. By adding hydrophobic sides using alkenyl succinic anhydrides such as octenyl succinic anhydride (OSA) and dodecenyl succinic anhydride, starch can be used to facilitate emulsion formation and stabilize emulsion (1).

OSA-modified starch is one of the most frequently used hydrophobically modified starches in the preparation of emulsions. In previous studies, OSA starches have been prepared using starches from different botanical sources including amaranth (2), arrowroot (3), banana (4), breadfruit (5), corn (2,6–12), kidney bean (12), lentil (4), quinoa (1), rice (12), sago (13,14), sorghum (15), sweet potato (4,12), and wheat (12). Nevertheless, very few studies on potatoes have been undertaken. Potato starch has various distinct properties, including high inherent viscosity, swelling ability, solubility, paste clarity, and a low tendency to retrograde. In comparison to the starches isolated from other plant sources, potato starch contains a high level of phosphate groups. Phosphate groups confer a polar character or negative charge, which can achieve partial water solubility for the fat globules and, thereby, could provide resistance to an emulsion network (16).

OSA-modified starch is produced through the esterification reaction between single-bond hydroxyl groups

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of starch and the carbonyl groups of OSA. The most commonly described synthesis pathway for OSA-modified starch is by reacting the starch in a mildly alkaline aqueous medium. The mild basic conditions reduce the hydrogen bonding between starch chains and induce the formation of alkoxide functionalities which consequently favors the substitution of hydrophobic octenyl groups (17). OSA starches can be effectively, irreversibly adsorbed at the interface of emulsions owing to their surface-active characteristics, forming a mechanical barrier that hinders the phase separation process (6). The degree of substitution (DS) is always used as an indicator to study the performance of OSA modification. A higher DS value has been associated with greater hydrophobicity and emulsifying capacity (18). OSA starch is currently used in a variety of food products, such as stabilizers and thickeners for soups, snacks, frozen or chilled foods, cloudy beverages, and juice concentrates (17).

OSA starch with modification up to 3% is legally permitted to be used as a food ingredient classified as modified starch (E1450) by U.S. Food and Drug Administration (FDA). OSA modification is often carried out in the aqueous slurry system, which sometimes reported to have low reaction efficiency and unevenly distributed octenyl groups, resulting in OSA starch with a low DS value (17). OSA modification of starch can be further increased through various pre-treatments. According to earlier research, starches modified by OSA with pre-treatments such as milling, hydrothermal, and enzymatic treatment showed higher emulsifying capabilities and stability than unpretreated OSA starch (7,19).

We hypothesized that application of single or dual pre-treatments with acid, dry heating, and acetylation could facilitate subsequent substitution of octenyl groups. Dry heat treatment could reveal the buried hydrophobic sites of starch (20). Meanwhile, the acetylation process can improve the hydrophobicity of starch granules by introducing hydrophobic acetyl groups (3). Besides, acid pre-treatment can also increase the substitution of octenyl groups by increasing the hydroxyl groups of starch through depolymerization of starch granules (21). Although numerous pre-treatment methods have been used to prepare OSA starch, to the best of our knowledge, little work has been reported on dry heating and acetylation pre-treatments. The studies on the effect of dual pre-treatment on subsequent OSA modification are also limited. Hence, the goal of the present work is to evaluate the effects of single and dual pre-treatments on the reactivity of the starch with OSA by assessing the physicochemical and functional properties of the resultant OSA starches.

2 Materials and methods

2.1 Materials

Potato starch was supplied by Deli Baking Ingredients Sdn. Bhd. (Penang, Malaysia). OSA (2-octen-1-ylsuccinic anhydride, 97%, mixture of cis and trans) and propylene oxide (99.5%) were purchased from Sigma Aldrich (Darmstadt, Germany), whereas acetic anhydride (99.5%) was bought from Merck (Darmstadt, Germany). All the chemicals and reagents used throughout this work were of analytical-grade quality.

2.2 OSA modification of potato starch via different pre-treatments

2.2.1 Single pretreatment by dry heating (D)

Starch (dry basis) was spread to a depth of 1–3 mm in opened aluminum moisture dishes, heated in an oven (120°C, 1 h 30 min), and cooled to ambient temperature (25°C) in a desiccator before use (20).

2.2.2 Single pretreatment by acetylation (C)

Starch (100 g, dry basis) was suspended in distilled water, and the pH of the slurry was adjusted to 8.0 using NaOH (1 M). The acetylation process was initiated by adding acetic anhydride (3 g) slowly to the reaction solution with constant stirring and the pH of the reaction slurry was maintained at 8.0–8.5. After 1 h, the acetylation process was terminated by adjusting the pH of the slurry to 4.5 using HCl (0.5 M), and the slurry was filtered, washed with distilled water, dried (40°C, 48 h), and grounded into powder (22). The acetyl group content value was then determined according to a titration method (22) (acetyl group content values: CO = 2.14%, ACO = 2.49%).

2.2.3 Single pretreatment by acid modification (A)

Starch (100 g, dry basis) was incubated in HCl (150 mL, 1.64 M) at 40°C for 4 h with continuous gentle stirring. After the pH was adjusted to 6.0 using NaOH (10%), the suspension was washed with distilled water, recovered by centrifugation (1,500 × *g*, 15 min), dried (40°C, 48 h), and ground into powder (23).

2.2.4 Dual pretreatments

Dual pretreatments were conducted by combining two pre-treatment methods. In this study, two combinations were created: [1] acid modification → dry heating and [2] acid modification → acetylation.

2.2.5 OSA modification

Native, single, and dual pretreated potato starches (50 g, dry basis) were dispersed in distilled water (adjusted to pH 7.6 using 3% NaOH) using an electrical stirrer fitted with a stainless-steel propeller. OSA modification was initiated by the addition of OSA (3% on the basis of the dry weight of starch), dropwise over a 1-h period, and the reaction was thought to be complete when the pH (7.6) of the reaction slurry remained constant (at least 15 min). After centrifuging at 1,500 × *g* for 15 min, the OSA-modified starch was washed twice with distilled water and once with citric acid (pH 4.5), dried (40°C, 48 h), and ground into powder (1). An acid–base titration method was adopted to measure the degree of octenyl group substitution (DS) (1).

2.3 Physicochemical properties

2.3.1 Oil-binding capacity (OBC)

Starch (1 g, dry basis) and canola oil (5 mL) were vortex-mixed for 1 min and kept at room temperature (25°C, 30 min) with intermittent shaking (5 s every 10 min). After subjecting to centrifugation (1,500 × *g*, 20 min), the unbound oil was decanted. The OBC was then computed as follows (Eq. 1):

$$\mathrm{OBC}(\%) = (W_\mathrm{o}/W_\mathrm{s}) \times 100\% \tag{1}$$

where W_o and W_s are the weight of the bounded oil (g) and the starch (g), respectively.

2.3.2 Water-binding capacity (WBC)

Starch (1 g, dry basis) and distilled water (30 mL) were vortex-mixed for 2 min. After subjecting to centrifugation (1,500 × *g*, 20 min), the unbound water was discarded and the wet precipitate was dried in an oven (40°C, 24 h) (14). The WBC was then calculated using the following equation (Eq. 2):

$$\mathrm{WBC}(\%) = [(W_\mathrm{w} - W_\mathrm{d})/W_\mathrm{s}] \times 100\% \tag{2}$$

where W_w, W_d, and W_s are the weight of wet precipitate (g), dried precipitate (g), and initial weight of the starch (g), respectively.

2.3.3 Particle size measurements

A particle size analyzer (Mastersizer 2000, Malvern Instruments Ltd., UK) was used to measure the starch particle sizes. The granularity analyzer was loaded with 0.5 g of starch sample, and the refractive index of 1.530 was used (13).

2.3.4 Scanning electron microscopy (SEM)

A field-emission, scanning electron microscope (Leo Supra 50 VP, Carl-Ziess SMT, Oberkochen, Germany) at an accelerating voltage of 5 kV was used to examine the morphological characterization of the starch granules. Starch granule (pre-dried at 40°C for 24 h) was scattered on an aluminum stub with double-sided carbon adhesive tape and sputter-coated with a layer of gold, using Polaron SC 515 Sputter Coater (Fison Instruments, VG Microtech, Sussex, UK).

2.3.5 Amylose content

The mixture of starch (0.1 g, dry basis) and dimethyl sulfoxide (8 mL, 90%) was heated in a water bath at 85°C with shaking. After cooling, the solution was initially diluted to 25 mL and further diluted (1:40) to 40 mL with deionized water, followed by the addition of iodine solution (5 mL, 0.002 $\mathrm{mol{\cdot}L^{-1}}$ iodine in 0.0065 $\mathrm{mol{\cdot}L^{-1}}$ potassium iodide) and brought to the final volume of 50 mL. After 15 min of incubation at 25°C, the absorbance of the solution at 600 nm was read using a spectrophotometer (UV-160A Shimadzu Corp., Kyoto, Japan), and the amylose content was determined from the calibration curve prepared earlier (14).

2.3.6 Pasting properties

Starch (2 g, 14% wet basis) was dissolved in distilled water (25 mL) in the aluminum RVA sample canister. Subsequently, the starch slurry was heated at 12°C·$\mathrm{min^{-1}}$ to 95°C, held for 2.5 min, and cooled to 50°C, with rotating speeds of 960 (first 10 s) and 160 rpm, in a rapid visco analyzer (model RVA-4 Series, Newport Scientific, Warriewood, Australia).

The pasting characteristics of the starch were then analyzed using the RVA software (Thermocline for Windows version 2.2).

2.4 Statistical analysis

The experimental results in the present work were processed using SPSS statistics software (version 22.0, IBM Corp., Armonk, NY, United States) and presented as mean ± standard deviation (SD) of three independent experiments. One-way analysis of variance, ANOVA, was performed and the difference among means was determined by Tukey's multiple comparison test at a 95% confidence level.

3 Results and discussion

3.1 Degree of OSA substitution (DS)

The degree of substitution (DS) is the average number of octenyl group-substituted hydroxyl groups per anhydroglucose unit in starch. It was evident that the OSA starch with a higher DS value could produce an emulsion with a higher resistance to droplet coalescence (18). The DS values of all the OSA starches with and without pre-treatments are presented in Table 1.

OSA starches that were pretreated using two treatments showed considerably ($P < 0.05$) higher DS values compared to those of single pre-treated and OSA starch without pre-treatment (O). Acid-modified-dry heated-OSA starch (ADO) had a significantly ($P < 0.05$) higher DS value compared to acid-modified-acetylated-OSA starch (ACO) at 0.015 and 0.010, respectively. For the single pre-treatment of the OSA starches, dry heated-OSA starch (DO) showed higher DS compared to the acetylated- and acid-modified-OSA starches. Through the removal of water, dry heating introduces vacant space or fine cracks that can facilitate deeper penetration of octenyl groups into the starch granule (24). By comparing to O, single pre-treatment of heat alone (DO) increased the DS by 50%, whereas ADO resulted in a 130.2% increment. Acid hydrolysis increased the formation of starch hydroxyl groups for the substitution of octenyl groups. Our findings are in agreement with the studies of Basilio-Cortes et al. (21) and Chung et al. (8), who also showed that the DS value of OSA starch could be increased through acid and dry heating pretreatments, respectively.

3.2 OBC

OBC represents the dry starch's capability to bind oil through capillary forces. Starch with a higher OBC has a higher adsorption efficiency and could provide a denser adsorbed layer surrounding the emulsion droplets, consequently imparting stability to the emulsion (20). The OBC of all the OSA starches is illustrated in Table 2. All pre-treatments rose the OBC of the OSA starch significantly ($P < 0.05$), with greater increment observed from the dual pre-treatments due to higher substitution of hydrophobic octenyl groups.

Among the single pre-treatments, the highest ($P < 0.05$) OBC was achieved with acid treatment (276%) followed by dry heating (252%) and acetylation (223%). Acid modification increases the free hydroxyl groups of starch that intensifies the OSA modifications resulting in increased

Table 1: Degree of OSA substitution (DS) of OSA starches

OSA starches	DS
O	0.006 ± 0.001^{a}
DO	0.009 ± 0.001^{b}
CO	0.008 ± 0.001^{ab}
AO	0.008 ± 0.001^{ab}
ADO	0.015 ± 0.001^{d}
ACO	0.010 ± 0.001^{c}

Data is expressed as mean ± standard deviation ($n = 3$).
$^{a-d}$ with different superscripts indicate the significant difference ($P < 0.05$) among starch sample.
O: OSA-modified; D: dry heat treated; C: acetylated; A: acid-modified.

Table 2: OBCs of OSA starches

OSA starches	OBC (%)
O	211 ± 1^{a}
DO	252 ± 2^{c}
CO	223 ± 2^{b}
AO	276 ± 1^{d}
ADO	280 ± 1^{e}
ACO	290 ± 1^{f}

Data are expressed as mean ± standard deviation ($n = 3$).
$^{a-f}$ with different superscripts indicate the significant difference ($P < 0.05$) among starch samples.
O: OSA-modified; D: dry heat treated; C: acetylated; A: acid-modified.

hydrophobicity. The starch granule-associated proteins that are weakly located in the channels and surfaces of the starches could expand and modify through dry heating, which creates binding sites for oil. Dry heating could also uncover the starch's hidden hydrophobic sites, thereby increasing starch's attraction to oil (20).

ACO had significantly ($P < 0.05$) higher OBC (290%) compared to ADO. The subsequent modifications by substitution of acetyl groups increased the affinity of starch to oil because of the hydrophobic property of the acetyl group (3,21). At the same time, the steric hindrance of the acetyl group obstructed the formation of hydrogen bonds between water molecules and free hydroxyl groups in starch, hence strengthening the OBC of the acid-modified starch (3). The occurrence of amylose leaching could also be the cause of the rise in hydrophobicity of starch following dry heating. The high heating temperature caused the water in the interior of starch granules to be lost, broke, and produced pores on the starch granules. Subsequently, the solubility of starch was increased and led to amylose leaching. The leached amylose will encircle the gelatinized starch granules and form a complicated matrix that could trap the oil (9).

3.3 WBC

To stabilize an emulsion, particles need to possess amphiphilic with both hydrophilic and hydrophobic properties. WBC describes the hydrophilicity and the ability of starch granules to absorb and retain both the inherent or added water. The WBC of all the OSA starches is presented in Table 3.

Interestingly, all pretreatments increased ($P < 0.05$) the hydrophilicity of the OSA starches compared to O although the degree of increment was much lesser compared to the increment of their hydrophobicity. The hydrophilic groups of OSA starch are more likely to be exposed after 2–4 h of dry heating (25). The glycosidic linkage between the starch molecules will be broken and hydrolyzed after being treated in an acid slurry. As a result, there will be more hydrophilic hydroxyl groups, which can hold onto and absorb more water molecules (26). Similarly, Koksel et al. (23) also found an increase in the WBC of starch after acid modification. The incorporation of large acetyl groups disrupted the inter- and intra-granular interactions within the starch and hence, making it easier for water to reach the amorphous area (3).

Table 3: WBCs of OSA starches

OSA starches	WBC (%)
O	110 ± 1^a
DO	129 ± 1^c
CO	115 ± 1^b
AO	128 ± 1^c
ADO	155 ± 2^e
ACO	137 ± 2^d

Data is expressed as mean ± standard deviation ($n = 3$).
$^{a-e}$ with different superscripts indicate the significant difference ($P < 0.05$) among starch sample.
O: OSA-modified; D: dry heat treated; C: acetylated; A: acid-modified.

Both of the dual pre-treated OSA starches had significantly ($P < 0.05$) higher WBC (137–155%) compared to those of single pre-treated (115–129%). Similar findings were seen in the DS values (Section 3.1), in which it was found that the DS values of OSA starches (0.010–0.015) treated with dual pre-treatments were considerably higher than those with single pretreatment (0.008–0.009), suggesting the dual pre-treatments are more effective than single pre-treatments at increasing the hydrophilicity of OSA starch. The incorporation of octenyl groups during OSA modification weakened the granular structure of starch, prevented starch chains from aligning, disrupted intermolecular hydrogen bonds between starch chains, and thereby, increased the tendency of starch to engage water (2).

3.4 Particle size

The formation of a stable emulsion is greatly influenced by the starch granule's particle size (26). Smaller starch particles are more liable to occupy the oil–water interface than bigger starch particles owing to their faster and stronger adsorption kinetics, which gives rise to higher emulsion stability (10). The particle sizes ($D[4,3]$) of OSA starches are listed in Table 4.

Among the single pre-treated OSA starches, only the one pre-treated with acid modification (AO) displayed a smaller particle size when compared to the control (O). Results revealed that acid modification had reduced the particle size of OSA starch at 3.5% through the cleavage of starch chains. When starch is subjected to acid modification, the crystalline portion of the starch granules will be transformed into starch crystals with smaller particle sizes whereas the amorphous portion will be hydrolyzed (10).

The particle size of OSA starch was increased by single pre-treatments of dry heating and acetylation, by

Table 4: Particle sizes ($D[4,3]$) of OSA starches

OSA starches	Particle size $D[4,3]$ (μm)
O	49.2 ± 0.5^{ab}
DO	60.7 ± 0.5^{c}
CO	52.4 ± 0.8^{b}
AO	47.5 ± 0.9^{a}
ADO	78.3 ± 1.1^{d}
ACO	58.6 ± 2.2^{c}

Data are expressed as mean ± standard deviation ($n = 3$).
$^{a-d}$ with different superscripts indicate the significant difference ($P < 0.05$) among starch samples.
O: OSA-modified; D: dry heat treated; C: acetylated; A: acid-modified.

23.5% ($P < 0.05$) and 6.6% ($P > 0.05$), respectively. The enlargement in the particle size of starch after dry heat treatment could be attributed to the particle aggregation as a result of enhanced hydrophobicity, as shown in Section 3.2. Our finding is similar to the findings of Diao et al. (11), who worked on dry-heated starches and also noted an increase in the particle size of starch after subjecting to dry heat treatment. The particle size of OSA starch did not change considerably with the single pre-treatment of acetylation, which may be related to their low acetyl group content of less than 5% (27). Ayucitra (22) also found no differences in the starch particle size after being modified by acetylation.

Acid modification followed by dry heating and acetylation further increased the size of OSA starches by 59.3% (ADO) and 19.1% (ACO), respectively. Although acid pretreatment alone can reduce the OSA starch particle size by chopping down the amorphous region, the depolymerization of the starch chains coupled with secondary pre-treatments extended the OSA modification (DS value), further causing starch to swell more and thereby increasing in particle size. The dry heating may facilitate more extension of chain length due to more starch hydroxyl groups being substituted by octenyl groups compared with acetylation as shown by higher DS in their single pre-treatment (Table 1) (10,13).

3.5 SEM

The SEM micrographs of all the OSA starch samples are presented in Figure 1. The surfaces of OSA starch granules (O) (Figure 1a) exhibited slightly rough surfaces with pores, demonstrating that the substitution of octenyl groups on the starch was surface-preferentially. Additionally, the finding also suggests that OSA modification did not damage the potato starch granules' morphological structure. Similar observations have been made by Remya et al. (4), who also worked on OSA-modified potato starch.

Dry heated OSA starch granules (DO) were observed to have bumpy surfaces and were irregularly shaped (Figure 1b). During the dry heating process, the water in the starch granules was fast evaporated. As a result, the structure and surfaces of the starch granules loosened and collapsed inward. On the surfaces of DO, some cavities, fractures, and fissures were visible. Dry heat treatment under extreme conditions (elevated temperature with low humidity) has led to the development of defects (28). Additionally, dry heat treatment caused the OSA starch granules to aggregate. The agglomeration of DO could be attributed to the adhesion of leached amylose or the hydrophobicity induced by dry heating. Our finding is consistent with the study conducted by Diao et al. (11).

The acetylation pretreatment (CO) had no effect on the shape and size of the OSA starch granules (Figure 1c). Nevertheless, some pits were observed at the surfaces of CO, implying that the acetylation process took place primarily at the surfaces of starch granules. Similarly, some pits were also seen on the acetylated potato starch of Cao and Gao (29). Besides, CO starch granules were found to be fused to one another. These could be attributed to the introduction of acetyl groups, hydrogen bonding interactions, and the surface gelatinization of acetylated starches.

The surfaces of AO (Figure 1d) were rougher with indentations, pits, and fractures than that of the counterpart (O). Similar to a previous study (21), the deformations on the surfaces of starch were thought to be due to surface erosion after being pre-treated with acid. AO starch granules also appeared to adhere to each other. Previous reports concluded that the adhesion of the starch granules was due to the ability of self-association of leached amylose. The leached amylose could form an amorphous polymer network that spreads over the neighboring granules, resulting in aggregated granules (26,30).

It is evident that both of the dual pre-treated OSA starch granules: ADO (Figure 1e) and ACO (Figure 1f) have undergone more extensive modifications and have higher granular deformation compared to other counterparts. These are in line with the results of the DS values for OSA starches (Section 3.1). The extent of the starch granules' deformation increases with increasing DS levels (21).

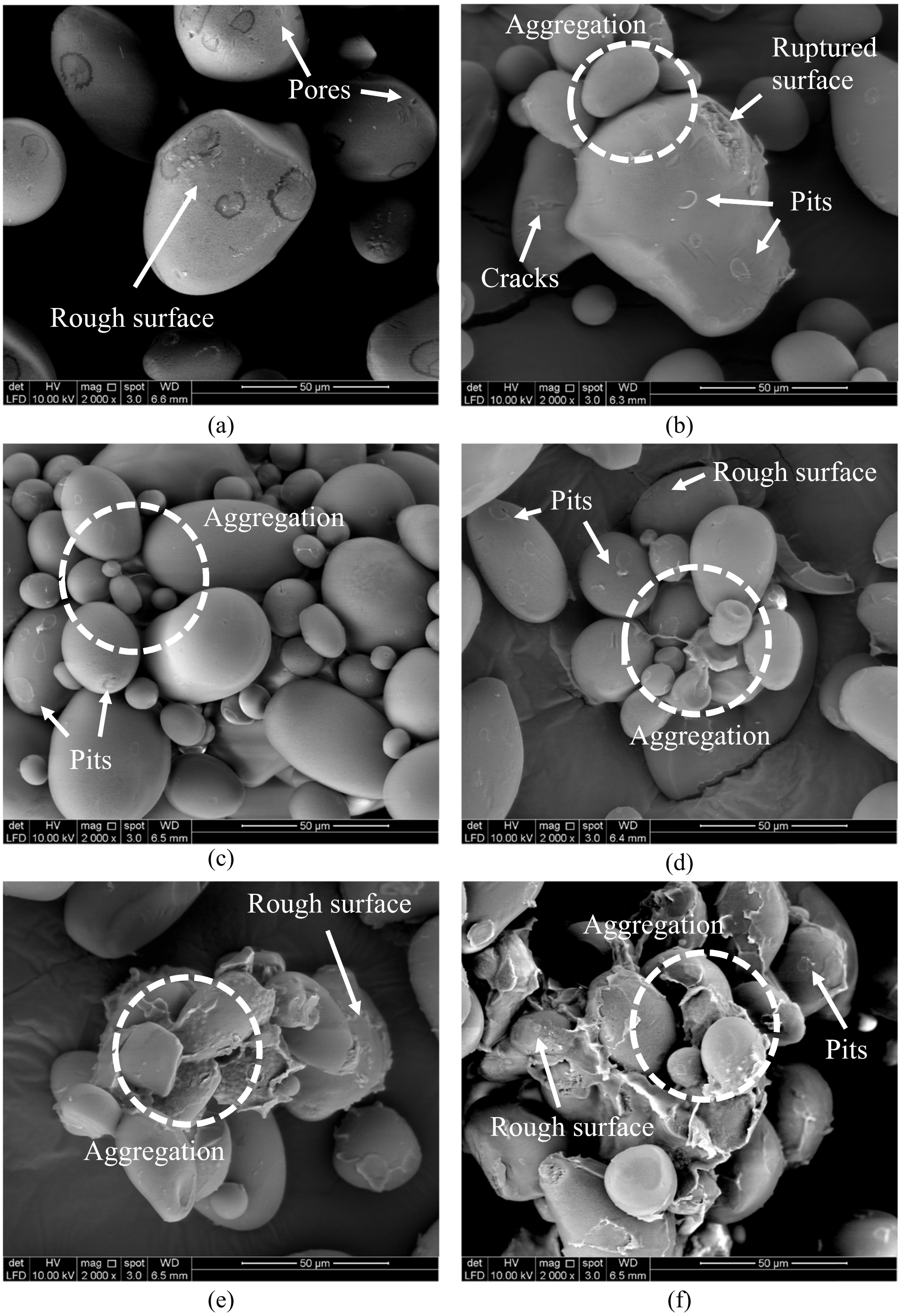

Figure 1: SEM micrographs (2,000 ×) of OSA starch granules where O – OSA-modified, D – dry heat treated, C – acetylated, A – acid-modified: (a) O, (b) DO, (c) CO, (d) AO, (e) ADO, and (f) ACO.

3.6 Amylose content

The amylose content was determined to investigate the relationship between starch amylose content and the impacts of pre-treatments on the subsequent OSA modification. The amylose contents of all the OSA starch samples are shown in Table 5.

Among all the pre-treatments, acid modification reduced the amylose content of OSA starch the most (for 66%) when compared to O. Acid predominantly penetrates and accesses

Table 5: Amylose contents of OSA starches

OSA starches	Amylose content (%)
O	36.4 ± 0.1^{e}
DO	20.9 ± 1.0^{d}
CO	17.0 ± 0.1^{c}
AO	12.3 ± 0.1^{b}
ADO	7.4 ± 0.4^{a}
ACO	8.5 ± 0.1^{a}

Data are expressed as mean ± standard deviation (n = 3).

$^{a-e}$ with different superscripts indicate the significant difference ($P < 0.05$) among starch samples.

O: OSA-modified; D: dry heat treated; C: acetylated; A: acid-modified.

the amorphous region of starch, which is primarily made up of amylose. The hydrolysis of the glycosidic linkages will result in a decrease in the molecular weight of the chains, which will lower the amount of amylose in the starch (31).

The amylose contents of dual pre-treated OSA starches (7.4–8.5%) were remarkably lesser ($P < 0.05$) than the OSA starches treated with single pretreatments (12.3–20.9%) and O (36.4%). This finding complies with our earlier results in Sections 3.1 and 3.5. Amylose and amylopectin are found in the amorphous and crystalline domains of starch granules, respectively. When compared to the crystalline domain, the amorphous domain is more readily accessible (14). As a result, the functional group is preferentially substituted and disrupts the amorphous domain more extensively compared to the crystalline domain (18).

As the DS values increased, the amylose content of OSA starches decreased. This finding implies that the amylose content reduction was correlated with the degree of OSA modification. Previous literature studies have also reported on the decrease in amylose content in starch after OSA modification (5,6,12).

3.7 Pasting properties

Table 6 shows the pasting properties of all the OSA and pretreated OSA starches. The temperature at which the viscosity starts to rise in response to the heating process is known as the pasting temperature (PT) (15). All dry heated OSA starches (DO and ADO) showed considerably lower ($P < 0.05$) PT than the unpretreated OSA starch (O), with a greater reduction observed in the single pretreated sample. The decrease in PT was attributed to the loosening of the interior structure of starch (Figure 1b) caused by the reorganization of the amorphous region and dissociation of the double helix structure of starch after dry heat treatment. The loosened granule structure in the dry heated starch made it easier to hydrate (8). The PT of OSA starch also decreased with the incorporation of acetyl groups. The interactions within the amorphous domains and the intermolecular hydrogen bonds of starch were disrupted by the inclusion of acetyl groups, thus decreasing the PT (31).

The maximum viscosity can be attained while heating is known as peak viscosity (PV) (32). Among the OSA starches, CO had the highest PV value ($P < 0.05$). The addition of acetyl groups interfered with the alignment of the starch chains due to steric hindrance, resulting in structural disorder and the development of a starch matrix with a more ordered structure, stronger resistance to deformation, and a higher PV (33). AO demonstrated the lowest PV value among all the OSA starch samples, and

Table 6: Pasting properties of OSA starches

OSA starches	Pasting properties			
	PT (°C)	BD (cP)	SB (cP)	PV (cP)
O	69.8 ± 0.4^{d}	3,403 ± 75^{d}	698 ± 66^{d}	9,016 ± 91^{e}
DO	63.8 ± 0.9^{a}	2,832 ± 20^{c}	394 ± 47^{c}	7,174 ± 19^{d}
CO	66.5 ± 0.5^{bc}	5,622 ± 32^{e}	306 ± 56^{bc}	10,396 ± 7^{f}
AO	68.9 ± 0.5^{d}	489 ± 5^{a}	70 ± 2^{a}	671 ± 9^{a}
ADO	66.0 ± 0.5^{b}	445 ± 3^{a}	215 ± 4^{b}	1,266 ± 21^{b}
ACO	67.8 ± 0.8^{cd}	1,483 ± 36^{b}	344 ± 10^{c}	2,573 ± 54^{c}

Data are expressed as mean ± standard deviation (n = 3).

$^{a-f}$ with different superscripts indicate the significant difference ($P < 0.05$) among starch samples.

O: OSA-modified; D: dry heat treated; C: acetylated; A: acid-modified; PV: peak viscosity; BD: breakdown viscosity; SB: setback viscosity; PT: pasting temperature.

this was attributed to its shorter chains obtained through acid depolymerization. The pastes of acid-modified starch were weak as their amorphous regions have been degraded by acid (21).

Breakdown viscosity (BD) is the difference between the peak and trough viscosity, and it reflects the degree of disintegration of swollen granules as well as the stability of starch paste under heating and shearing (15,32). All of the OSA starches that have been pre-treated, except CO, exhibited considerably lesser ($P < 0.05$) BD than O, demonstrating that the treatments increased the thermal and shear stability of OSA starch. When compared to O, acid pre-treatment, either alone (A) or accompanied by dry heat treatment (AD), reduced BD the greatest. Both the acid modification and dry heating caused the internal realignment within the starch granule, thereby conferring OSA starch heat and shear resistances. The enhancements in the starch stability upon heat and shear after acid and dry heat treatments have also been reported previously (8,23). Single pretreatment of acetylation (C) increased the BD by 66% and produced OSA starch with the highest ($P < 0.05$) BD or lowest resistance against heat and shear among all the OSA starch samples. The addition of acetyl groups weakened and degraded the formation of inter- and intra-molecular hydrogen bond structures in the starch granule, the molecular structure, thus resulting in a drop in PT but a rise in BD (34).

Starch has a strong tendency to retrograde when being used as an emulsion stabilizer, which usually results in emulsion instability (10). Setback viscosity (SB) measures the extent of reassociation of leached amylose upon cooling, and it is often used to evaluate the tendency of starch retrogradation (32). When compared to pre-treated OSA starches (70–394 cP), O had a greater SB (698 cP), implying that the retrograde tendency of OSA starch was lowered by the pretreatments (2). Among all samples, AO showed the lowest ($P < 0.05$) SB, demonstrating the least vulnerability against retrogradation. Short-chain amyloses in acid-modified starch are less likely to reassociate. Furthermore, the introduction of octenyl groups substituting the hydroxyl groups on starch could create electrostatic hindrance, subsequently limiting the formation of hydrogen bonds and the reassociation of amylose chains (35).

4 Conclusions

Acid modification followed by dry heating (A → D) is the most effective pretreatment in improving the subsequent OSA modification. While all the pretreatments were observed to enhance the OBC and WBC of OSA starches, acid modification followed by acetylation (A → C) produced OSA starch with highest hydrophobicity whereas acid modification followed by dry heating (A → D) produced OSA starch with the highest hydrophilicity. The starch particle size was only reduced by a single pretreatment of acid modification (A), whereas other pretreatments expanded it. The morphology of OSA starch was affected by all pre-treatments to some degree, but dual pre-treatment gave more granular deformation. Dual pre-treatments also reduced the amylose content of OSA starch more than those single pre-treatments. As shown by the paste viscosity test results, the resistance of OSA starch's against retrogradation was greatly increased by all of the pretreatments. In short, the dual pretreatments were found to significantly modify the OSA starch, in terms of their physicochemical characteristics. OSA starches with dual pre-treatments are expected to possess a greater ability to act as a stabilizer for emulsions since they showed higher DS, OBC, and WBC than those of single pre-treated and OSA starch without pretreatment. This study could serve as a basis for future studies in the emulsion.

Abbreviations

A	acid modification
ACO	acid-modified-acetylated-OSA starch
ADO	acid-modified-dry heated-OSA starch
AO	acid-modified-OSA starch
C	acetylation
CO	acetylated-OSA starch
D	dry heating
DO	dry heated-OSA starch
O	OSA modification/OSA-modified starch without pre-treatment
OSA	octenyl succinic anhydride

Funding information: Ministry of Higher Education Malaysia for Fundamental Research Grant Scheme with Project Code: FRGS/1/2020/STG04/USM/03/1.

Author contributions: Xiao Xian Lim: writing – original draft, writing – review and editing, methodology, formal analysis; Nor Shariffa Yussof: writing – review and editing, Musfirah Zulkurnain: writing – review and editing, Uthumporn Utra: writing – review and editing, project administration.

Conflict of interest: The authors state no conflict of interest.

Data availability statement: All data generated or analyzed during this study are included in this published article.

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