

# Atmospheric pressure plasma jet pretreatment to facilitate cassava starch modification with octenyl succinic anhydride

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## ABSTRACT

Cassava starch (CS) was pretreated with atmospheric pressure plasma jet (APPJ), followed by esterification with octenyl succinic anhydride (OSA). This study was the first report investigating the effect of APPJ on CS modification with OSA. Results showed that APPJ pretreatment could change the morphological characteristics and crystallinity of CS. Consequently, the degree of substitution and reaction efficiency significantly improved compared with the untreated CS ( $P < 0.05$ ). In Confocal laser scanning microscopy, the fluorescence intensity of OSA-modified CS pretreated with APPJ for 10 min and 15 min was higher than those pretreated with APPJ for 1, 3, and 5 min. The onset temperature and enthalpy ( $\Delta H$ ) of native starch decreased after APPJ pretreatment and further decreased by OSA modification. APPJ–OSA–CS also showed better emulsion stability and emulsion activity. This study demonstrated that APPJ could be used as a novel approach to facilitate starch modification with OSA.

## 1. Introduction

Natural starch is limited in function due to its weak structure, which hinders its further application in most industries (Oderinde, Ibikunle, & Bakre, 2020). As such, physical, chemical, and enzymatic modifications have been widely explored to overcome these shortcomings and improve the physicochemical properties of starch (Punia, 2020; Xu et al., 2021). An example of chemical modification involves the use of OSA to modify starch; in this reaction, esterification occurs between starch and OSA. After esterification, hydrophilic starch molecules obtain surface-active properties by introducing hydrophobic groups from OSA groups. OSA-modified starch is mostly used to stabilize oil-in-water or water-in-oil emulsions for cosmetics, foods, and pharmaceuticals, such as salad dressings, beverages, processing aids, flavor-encapsulation agents, clouding agents, lotions, and body powders (Bajaj, Singh, & Kaur, 2019). Nowadays, 3% OSA (dry weight of starch) is permitted to modify starch

to obtain food grade products in many countries (Qian et al., 2019).

Starch and OSA are usually synthesized in a mild alkaline aqueous medium. However, OSA has low solubility in water, and OSA exists in the form of insoluble oil droplets or a small amount of dissolved OSA mixture in an aqueous phase; as a result, a heterogeneous reaction and a low reaction efficiency are obtained (Shogren, Viswanathan, & Felker, 2000). Several starch pretreatment methods, including dynamic high-pressure microfluidization (Li et al., 2018), ball milling (Li et al., 2017), ultrasound (Zhang et al., 2019), hydrothermal (Chen, He, & Huang, 2014), hydrothermal–alkali (Wang, Li, & Wang, 2017), high-speed shear (Wang et al., 2015), ionic liquids or other solvents systems (Neumann, Wiege, & Warwel, 2002; Li, Zhang, & Tian, 2016; Wang et al., 2020), and heat–moisture (Jiranuntakul, Pancha-arnon, & Uttapap, 2014) approaches, have been used to enhance reaction efficiency. In addition to improve the degree of substitution of OSA starch, these pretreatment methods could also have an impact on the other properties

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of OSA modified starch. Compared with starch modified by OSA without dynamic high-pressure microfluidization (DHPM) and hydrothermal pretreatment, DHPM-OSA-starch and H-OS-starch exhibited lower pasting temperature, higher peak viscosity (Chen, He, & Huang, 2014; Li et al., 2018). DHPM-OSA-starch also presented better emulsification performance (Li et al., 2018). Unlike conventional OSA modified starch, starch modified by OSA with ball milling pretreatment exhibited relatively low rigidity and viscosity as well as paste stability (Li et al., 2017). The OSA starch modified after hydrothermal-alkali (HA) presented higher full width at half maximum (FWHM) in contrast to normal OSA starch, indicating that the disruption of the short-range molecular order in HA-OSA-starch was more serious (Wang, Li, & Wang, 2017). OSA starch prepared using high speed shear exhibited higher freeze-thaw stability and paste clarity, higher stability of hot and cold paste, and lower pasting temperature compared with the control OSA starch (Wang et al., 2015). The average molar masses of the prepared starch esters using various carboxylic acid imidazolides as the reaction system were approximately five times that of the acyl chloride method (Neumann, Wiege, & Warwel, 2002). In addition, the emulsion, pasting, and textural properties of OSA-starch using ionic liquids as the reaction system were enhanced greatly (Li, Zhang, & Tian, 2016; Wang et al., 2020).

Atmospheric pressure nonthermal plasma (APNTP), which is also called “cold plasma,” is a kind of partial gas that is slightly ionized (<1%) (Wu, Sun, & Chau, 2018). The energy of this ionized gas is mainly stored in free electrons, and the overall temperature is kept at a low level. Several electrical discharges, including atmospheric pressure plasma jet (APPJ), corona discharge, dielectric barrier discharges, and microhollow cathode discharge (Nehra, Kumar, & Dwivedi, 2008; Lu, Cullen, & Ostrikov, 2016), are utilized to generate APNTP. Currently, APPJ is the most commonly used approach in practical applications owing to its simple design, energy-saving properties, and easy maintenance (Scholtz, Pazlarova, & Souskova, 2015). Previous studies have reported that the microstructure of starch granules can be changed remarkably after treatments with APNTP (Bie et al., 2016; Banura, Thirumdas, & Kaur, 2018). However, studies have yet to determine whether plasma-induced morphological changes facilitate cassava starch (CS) modification with OSA.

In this study, CS was pretreated with APPJ for different durations, followed by modification with OSA. The influence of APPJ on the subsequent OSA modification and the physicochemical properties of OSA-modified cassava starch were studied. This research proposed a novel method to facilitate starch modification with OSA, which has an excellent broad application prospect in food and other industries.

## 2. Materials and methods

### 2.1. Materials

The following materials were used in this study: CS (Xinxiang Liangrun Whole Grain Food Co., Ltd., Xinxiang, Henan, China) with 12.70% moisture content, 25.90% amylose content, and 0.71% protein content; OSA (97% purity, Sigma-Aldrich Chemical Co., St. Louis, MO, USA); and rapeseed oil (Zhejiang Xinshi Grease Co., Ltd., Huzhou, Zhejiang, China). Other chemical reagents were of analytical grade.

### 2.2. APPJ pretreatment of cassava starch

In this procedure, 30 g of CS was dispersed in 70 mL of distilled water to form a starch concentration of 30% (w/v) before treating with an APPJ (Tonson Tech Automation Equipment Co., Shenzhen, China) for 1, 3, 5, 10, and 15 min. Fig. S1 showed the schematic of APPJ setup. Plasma was induced by providing high frequency and high input power to the gas at atmospheric pressure. The input power to generate plasma was 650 W, and the height of the probe to a starch slurry was 8 cm. Starch slurries were stirred using a magnetic stirrer to prevent

sedimentation. After plasma pretreatment, the starch suspension was then centrifuged at 7,000 r/min for 5 min and the starch sediment was dried at 40 °C for 2 days. The samples were pulverized and sifted by a 100-mesh sieve.

### 2.3. Preparation of OSA-modified cassava starch

Native starch and APPJ-pretreated CS were modified with OSA as previously described (Ruan, Chen, & Fu, 2009) with slight modifications. Native starch and APPJ-pretreated CS were added to distilled water to obtain a 35% suspension with agitation. 3% NaOH solution was added to the starch suspension to adjust the pH to 8.0 with a pH meter (Mettler Toledo FE20). Then, 3% OSA (dry weight of starch) dissolved in absolute alcohol (1:5, v/v) was added to the starch suspension slowly within 2 h. The esterification was carried out at pH 8.0 ± 0.1 and 35 °C for 3 h. Afterward, 3% HCl was added to the reaction solution to adjust the pH to 6.5. After centrifugation, the primary product was washed three times with distilled water and 70% aqueous alcohol. After the specimen was dried at 40 °C for 2 days, the obtained starch granules were ground and passed through a 100-mesh sieve.

### 2.4. Determination of the degree of substitution (DS) and reaction efficiency (RE)

The DS of OSA-modified cassava starch was determined as previously described (Wang, Li, & Wang, 2017) with minor modification. In particular, a higher concentration of HCl-isopropanol solution (2.5 M instead of 0.2 M) and a lower volume of HCl-isopropanol solution (20 mL instead of 125 mL) were used.

The following equation was used to calculate the DS of OSA-modified cassava starch:

$$DS = \frac{0.162 \times (A \times M)/W}{1 - [0.209 \times (A \times M)/W]} \quad (1)$$

where A refers to the titration volume of NaOH solution (mL), W represents the dry weight (g) of the OSA starch, and M represents the molarity of NaOH solution.

RE was calculated as follows:

$$RE = \frac{\text{Actual DS}}{\text{Theoretical DS}} \times 100\% \quad (2)$$

### 2.5. Scanning electron microscopy

Before observation, the samples were placed on a metal sample holder and coated with a gold layer. Images of cassava starch particles were taken by using a scanning electron microscope (SEM; SU8010, Hitachi Int., Japan) operated at 3 kV. Images of starches were obtained at 3000 × Magnifications.

### 2.6. X-ray diffraction

The relative crystallinity and crystalline structure of the starch samples were determined through an X-ray diffractometer (XRD; Bruker D8 ADVANCE). The scanning region of the diffraction angle was from 5° to 60° with a rate of 2°/min. Relative crystallinity (%) was calculated using a previous method (Wang, Li, & Wang, 2017) by using JADE 6.0.

### 2.7. Fourier transform infrared spectroscopy

Changes in the chemical bonds of starch samples were evaluated through Fourier transform infrared (FTIR) spectroscopy (Bruker VERTEX70). Native and APPJ pretreated starch dried at 100 °C for 8 h to reach constant weight. Before measurement, 1 mg of starch samples were mixed with 100 mg of KBr and pressed into tablets. Then, the prepared tablets were scanned 32 times in the range of 400–4000 cm<sup>-1</sup>.

## 2.8. Confocal laser scanning microscopy analysis

OSA-modified starch and APPJ-pretreated starch combined with OSA-modified starch samples were stained with a dye for confocal laser scanning microscopy (CLSM). In brief, 0.5 g of the specimens was dissolved in 30 mL of deionized water. After adjusting the pH of the above mixtures to 8.0, methylene blue (MB<sup>+</sup>) solutions (1%) were added to each modified starch. Then, the resulting mixture was incubated with a shaker for 5 h at room temperature and washed with methanol to remove the residual dye. The fluorescence signal of the dye-stained starch samples was determined by a TCS SP8CLSM (Leica, Wetzlar, Germany). Leica objective lenses were 40 × 0.85, and excitation wavelength was set as 488 nm.

## 2.9. Differential scanning calorimetry

Differential scanning calorimetry (DSC; Mettler Toledo DSC 3) was used to determine the thermal characteristics of the starch samples in accordance with previously described methods (Wang, He, & Fu, 2015). In brief, 3 mg of the starch sample was added to an aluminum pan, and 9 μL of deionized water was added to make the starch sample to come in full contact with water. The aluminum pan was equilibrated overnight at room temperature. The starch samples were heated from 40 °C to 100 °C at 10 °C/min, and an empty pan was used as a reference.

## 2.10. Emulsification properties

### 2.10.1. Preparation of starch sample emulsions and physical stability of emulsions

In this procedure, 1 g of starch sample was weighed accurately and added to 100 mL of distilled water. Then, the starch slurry was boiled while being stirred with a magnetic stirrer for 20 min. Afterward, the solution was cooled to room temperature, and 30 mL of the paste solution and 10 mL of rapeseed oil were mixed. The mixture of above solution was sheared with a high-shear mixer (Ultraturax T25, IKA, Germany) at 12,000 rpm for 1 min. Coarse emulsions were further homogenized by a high-pressure microfluidizer (110P, Genizer, USA) at 5,000 psi for two cycles. 5 mL of the prepared emulsions was taken into the centrifuge tube and then centrifugated at 3000 rpm for 4 min to check its physical stability.

### 2.10.2. Determination of emulsifying activity index (EAI) and emulsion stability index (ESI)

The determination of emulsifying properties was based on previously described methods (Xu, Wang, & Fu, 2018) with slight modifications. 50 μL of emulsion was diluted 100 times with sodium dodecyl sulfate (SDS) solution (0.1%, w/w), and absorbance was measured with a Biotek microplate reader (Winooski, VT, USA) at 500 nm. Afterward, 0.1% SDS was recorded as a blank. EAI and ESI were obtained with the following equations:

$$EAI = \frac{2 \times 2.303 \times A_0 \times N}{C \times \Phi \times 1000} \quad (3)$$

and

$$ESI = \frac{A_0 * 10}{A_0 - A_{10}} \quad (4)$$

where  $A_0$  and  $A_{10}$  are recorded as absorbances at 0 and 10 min, respectively;  $N$  represents the dilution factor;  $C$  represents the concentration of starch (g/mL); and  $\Phi$  refers to the volume fraction of rapeseed oil.

### 2.10.3. Determination of zeta potential

The emulsion samples were diluted 1,000 times, and the zeta potential of the diluted emulsions was analyzed through Zetasizer Nano ZS90 (Malvern Instruments Ltd., Worcestershire, UK).

### 2.10.4. Determination of storage stability

The prepared emulsions were stored away from light at 25 °C. The stratification of these samples was observed on days 1, 3, 5, 7, and 9. The particle size of the samples was analyzed through Zetasizer Nano ZS90 (Malvern Instruments Ltd., Worcestershire, UK).

## 2.11. Statistical analysis

SPSS 22.0 was used for statistical analysis. Significant differences between means were determined with Duncan's multiple range tests at  $P < 0.05$ . The results were reported as mean ± SD.

## 3. Results and discussion

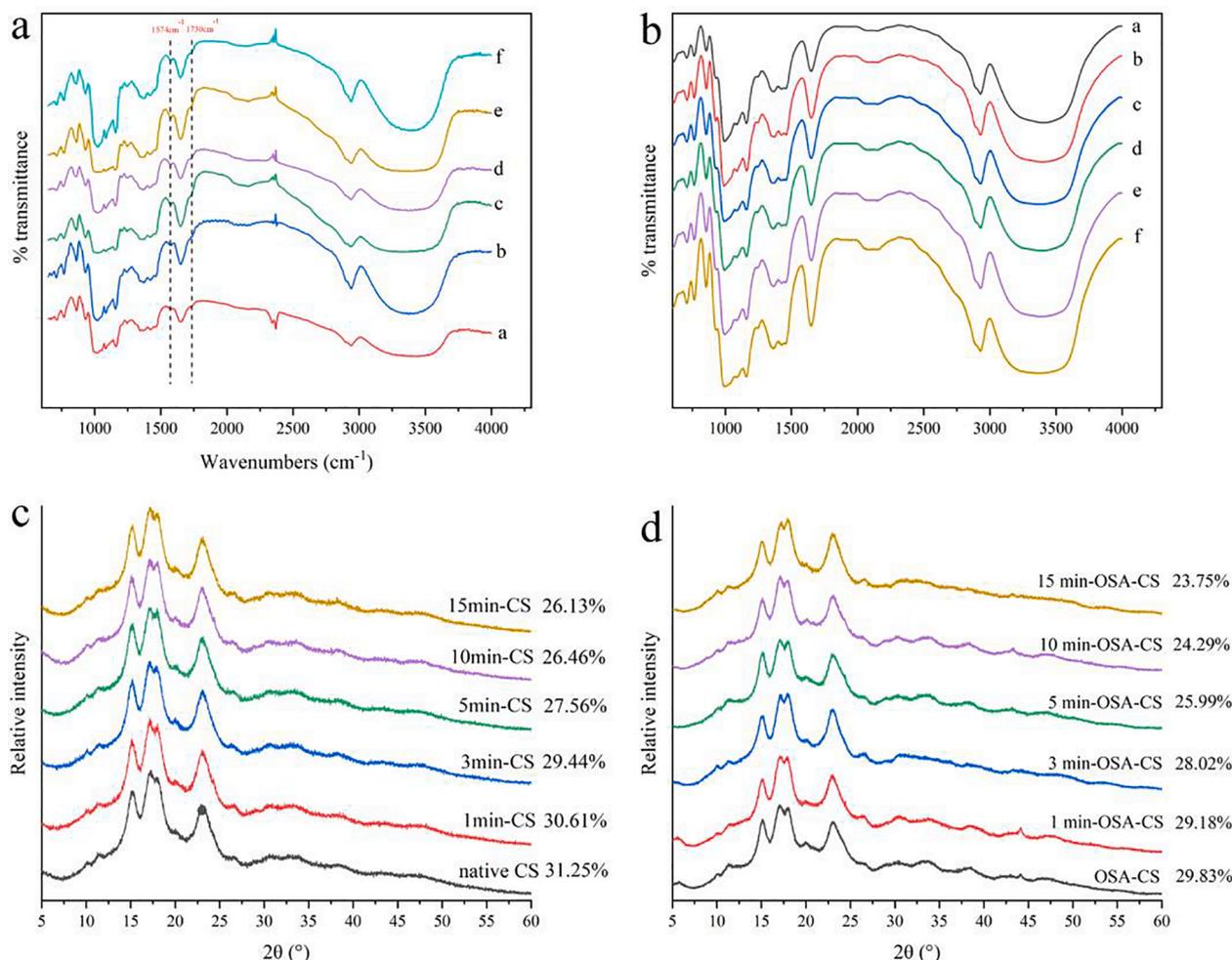
### 3.1. APPJ pretreatment for the OSA modification of cassava starch

#### 3.1.1. FTIR spectroscopy

FTIR spectroscopy was conducted to analyze the structure of each sample and investigate whether the substitution of carbonyl groups of OSA with hydroxyl groups occurred after esterification. As shown in Fig. 1a and Fig. 1b, all the starch samples had similar FTIR spectra. However, two new absorption peaks could be observed in the OSA-modified starches at 1574 and 1730  $\text{cm}^{-1}$  compared with those of native CS. The absorption peak located at 1574  $\text{cm}^{-1}$  was attributed to the asymmetric stretching vibration of the carboxylate group ( $\text{RCOO}^-$ ) (Miao et al., 2014). Another band at 1730  $\text{cm}^{-1}$  was assigned to the stretching vibration of carbonyl groups ( $\text{C=O}$ ) (Li, Zhang, & Tian, 2016). The above results indicated the OSA groups were successfully attached to the cassava starch granules after esterification. Fig. 1b exhibited the FTIR spectra of native and APPJ pretreated starches. After APPJ pretreatment, the position of these peaks of native starch did not change, but the width and height were transformed. The strong absorption peak located at 3392  $\text{cm}^{-1}$  was considered as the O—H stretch of starch (Bai, Shu, & Zhang, 2009). The peak height and width of APPJ pretreated starch at 3392  $\text{cm}^{-1}$  was slightly increased in contrast to native starch. This result was similar to previous studies (Thirumdas et al., 2017a; Yan et al., 2018). Thirumdas et al. (2017b) reported the phenomenon attributed to the incorporation of O—H groups by cleaving C—O—C glycosidic bonds after cold plasma treatment. Therefore, native cassava starch presented more hydroxyl groups after APPJ pretreatment.

#### 3.1.2. DS and RE

APPJ pretreatments remarkably and differently affected the DS and RE of the OSA modification of CS. As shown in Table 1, in contrast to starch without pretreatment, the starch pretreated with APPJ for 15 min was modified with enhanced DS (0.01197 vs. 0.01667) and RE (51.73% vs. 72.03%). The DS of OSA-CS improved by 39.23%. As the duration of APPJ pretreatment was extended from 1 min to 10 min, the DS and RE of OSA-modified cassava starch improved significantly ( $P < 0.05$ ). However, no significant difference was found between starches pretreated with APPJ for 10 and 15 min. High-speed shear pretreatment (Wang et al., 2015), hydrothermal pretreatment (Chen, He, & Huang, 2014), and heat-moisture pretreatment (Jiranuntakul, Pancha-arnon, & Uttapap, 2014) enhanced DS by 10.99%, 15.85%, and 17.53%, respectively, which were lower than those of APPJ pretreatment. However, the degree of the improvement of DS through dynamic high-pressure microfluidization pretreatment (Li et al., 2018) was higher than that through APPJ pretreatment. The DS of OSA was limited because of the immobility of the hydroxyl group on starch, so esterification was dependent on the availability of OSA distributed around starch molecules to obtain a high substitution rate. Thus, the modification of the CS structure via APPJ treatment might possibly cause an increasing probability of introducing OSA groups to starch molecules in this study. As such, this study also investigated the influence of APPJ on the structure of CS.



**Fig. 1.** (a) FTIR spectra of OSA-modified starches: (a) OSA-starch, (b) 1 min-OSA-starch, (c) 3 min-OSA-starch, (d) 5 min-OSA-starch, (e) 10 min-OSA-starch, and (f) 15 min-OSA-starch. (b) FTIR spectra of native and APPJ pretreated starches: (a) native cassava starch, (b) APPJ-treated CS for 1 min, (c) APPJ-treated CS for 3 min, (d) APPJ-treated CS for 5 min, (e) APPJ-treated CS for 10 min, (f) APPJ-treated CS for 15 min. (c) XRD of native CS and APPJ-treated CS for 1, 3, 5, 10, and 15 min (1 min-CS, 3 min-CS, 5 min-CS, 10 min-CS, and 15 min-CS, respectively). (d) XRD of OSA-CS, 1 min-OSA-CS, 3 min-OSA-CS, 5 min-OSA-CS, 10 min-OSA-CS, and 15 min-OSA-CS.

**Table 1**

Degree of substitution (DS) and reaction efficiency (RE) of OSA-modified cassava starch (CS) at different times after APPJ pretreatment.\*

Starch sample	DS × 10 <sup>4</sup>	RE (%)
OSA-CS (control)	119.72 ± 2.57 <sup>a</sup>	51.73 ± 1.11 <sup>a</sup>
1 min-OSA-CS	128.70 ± 7.22 <sup>b</sup>	55.61 ± 1.56 <sup>b</sup>
3 min-OSA-CS	138.25 ± 5.89 <sup>c</sup>	59.74 ± 1.27 <sup>c</sup>
5 min-OSA-CS	155.38 ± 3.44 <sup>d</sup>	67.14 ± 1.48 <sup>d</sup>
10 min-OSA-CS	165.93 ± 6.01 <sup>e</sup>	71.70 ± 2.60 <sup>e</sup>
15 min-OSA-CS	166.69 ± 3.19 <sup>e</sup>	72.03 ± 1.37 <sup>e</sup>

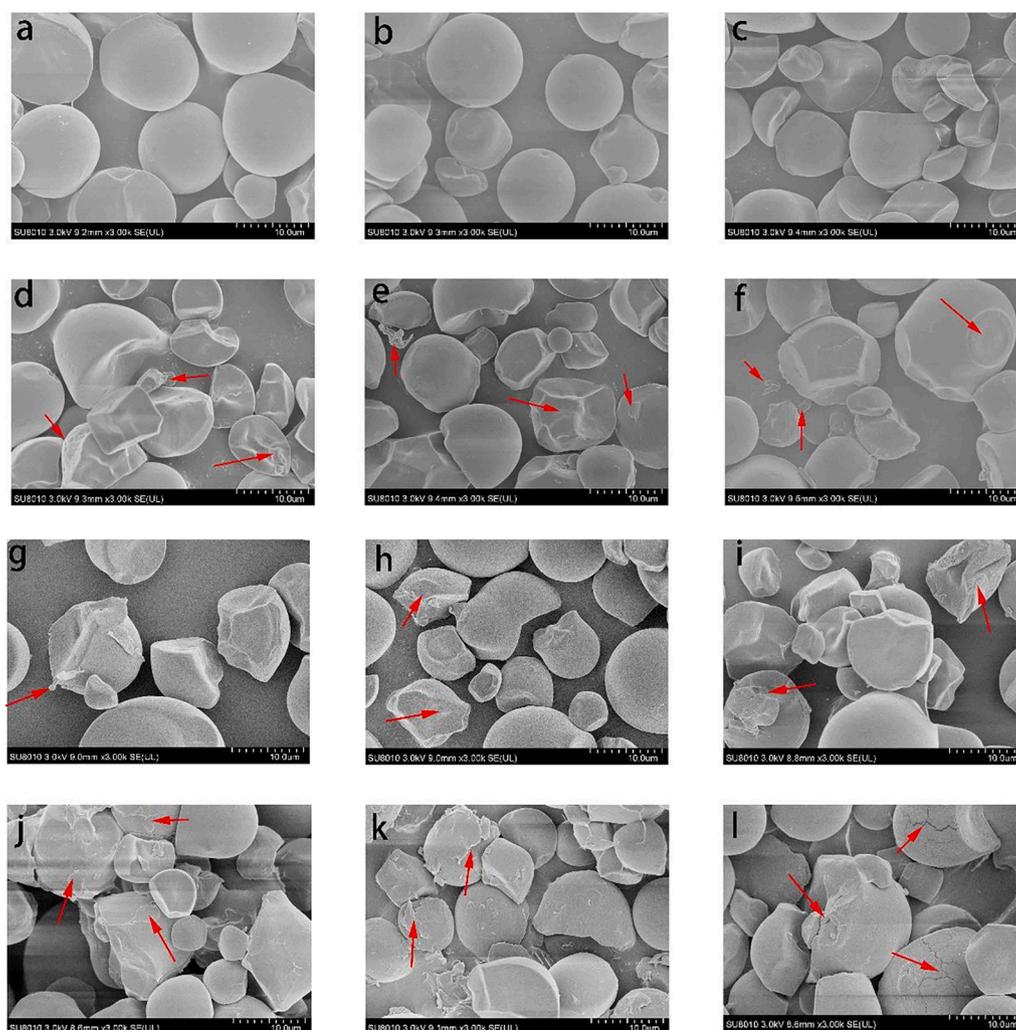
\* Values presented as the mean of triplicate ± standard deviation. Values with the same letters in the same column are not significantly different ( $P < 0.05$ ).

### 3.2. Changes of cassava starch structure after APPJ pretreatment and OSA modification

As shown in Fig. 2, APPJ pretreatment and OSA modification could change the surface morphology of CS. Consistent with previous results (Zhang et al., 2019), our findings showed that native CS particles were spherical, smooth, and irregularly shaped (Fig. 2a). No obvious change could be observed in the surface and shape of CS after APPJ pretreatment for 1 min. However, when starch was treated with APPJ for 3 min, some round starch particles were broken into small irregular fragments.

After APPJ pretreatment for 5, 10 and 15 min, starch was severely deformed, most CS particles appeared as tiny clumps, and the starch surface became rough. Similar results were reported after starch was treated with cold plasma in previous studies (Bie et al., 2016; Wu, Sun, & Chau, 2018). The APPJ treatment of starch could change the shape and surface morphology of cassava starch granules; consequently, the surface area of cassava starch granules and the contact with OSA during the reaction increased. The increased surface area of CS granules was caused by the plasma etching (Banura et al., 2018). These events could lead to a high collision tendency and an increase in the formation of products. After OSA esterification, all samples exhibited slightly rough surfaces and their edges also lost some definition. In general, as the degree of OSA substitution increased, the starch surface was damaged to a greater extent. The surfaces of 10 min-OSA-starch and 15 min-OSA-starch were most severely damaged (Fig. 2k and Fig. 2l). Some cracks even appeared on the surface of 15 min-OSA-starch, indicating that the esterification reaction may not only occur on the surface, but part of OSA could enter the starch cracks, thus improving the degree of starch substitution.

The crystalline structures of native CS and APPJ-pretreated starch were detected through XRD. Fig. 1c illustrates the relative crystallinity and XRD patterns of all the samples. Four strong diffraction peaks at 15°, 17°, 18°, and 23° could be observed in CS and APPJ-pretreated starch, which is a typical A-type polymorph (Xu, Chen, & Luo, 2019). The results suggested that the crystalline pattern of native CS remained



**Fig 2.** Scanning electron micrographs of native and OSA modified cassava starch. Native cassava starch (a), APPJ-treated CS for 1 min (b), APPJ-treated CS for 3 min (c), APPJ-treated CS for 5 min (d), APPJ-treated CS for 10 min (e), APPJ-treated CS for 15 min (f), OSA-starch (g), 1 min–OSA–starch (h), 3 min–OSA–starch (i), 5 min–OSA–starch (j), 10 min–OSA–starch (k), and 15 min–OSA–starch (l). Each sample was taken at  $3000\times$ .

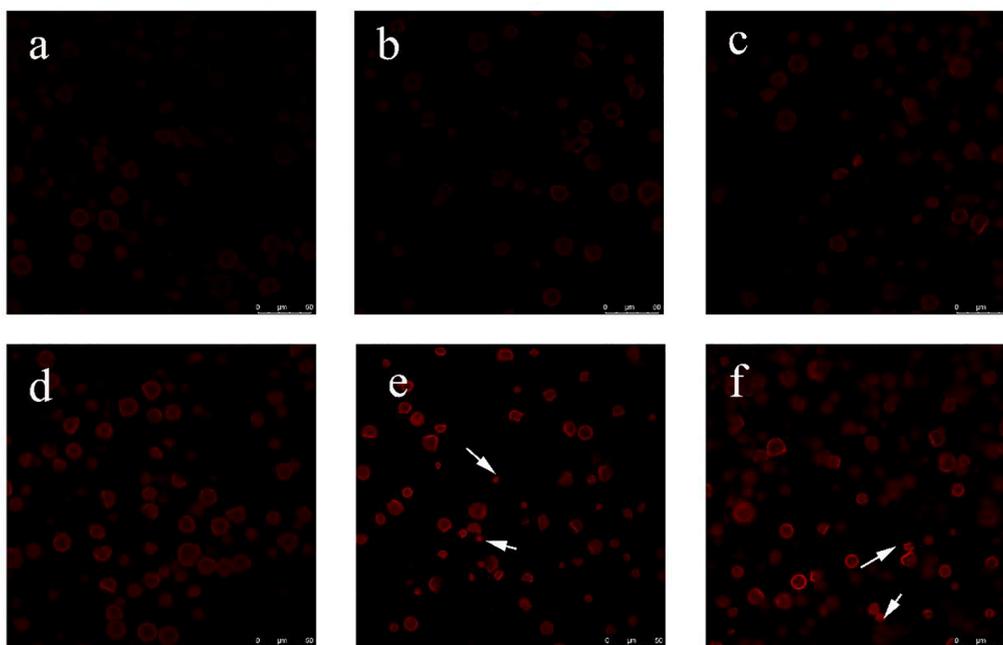
unchanged through APPJ pretreatment. The degree of crystallinity of native CS was 31.25%, and the relative crystallinity of APPJ-pretreated starch decreased from 30.61% to 26.13% from 1 min to 15 min. The decreased degree of crystallinity of native CS could be attributed to the interactions of the CS molecules and reactive species from APPJ, resulting in molecular scission (Zhu, 2017). The decrease in the degree of crystallinity could also be attributed to the depolymerization of the double helix structure of the starch molecule (Zhu, 2017; Zhang et al., 2019). As shown in Fig. 1d, the intensity of peaks at the  $2\theta$  values of  $15^\circ$ ,  $17^\circ$ ,  $18^\circ$ , and  $23^\circ$  were still strong after starch modified by OSA. However, there was a small decline in the degree of crystallinity of OSA-CS and APPJ–OSA–CS compared with the corresponding native and APPJ starch samples. This result illustrated that OSA modification did not change the crystalline pattern of native and APPJ pretreated CS, although the granules of these samples have been damaged to some extent.

The SEM and XRD results indicated APPJ could change the surface structure of CS and reduce the degree of crystallinity of CS. As a consequence, more hydroxyl groups of CS were exposed. These results can enhance the reaction probability between starch and OSA groups. Therefore, DS and RE increased as the duration of APPJ pretreatment was extended from 1 min to 15 min.

### 3.3. Characteristics of OSA-modified cassava starch

#### 3.3.1. Distribution of OS groups in starch granules

CLSM is widely adopted to investigate fluorescence intensity and the distribution of starch granules after the OS groups of starch are stained with  $MB^+$  dye (Chen, He, & Huang, 2014; Lu et al., 2016).  $MB^+$ , as a fluorescence dye that highlights anionic substances, can specifically label  $-COO^-$  of OS groups. Fig. 3 exhibited the CLSM optical sections of OSA-modified CS. No fluorescence could be observed in the native CS granules (data not shown), whereas fairly uniform and bright fluorescence could be detected in OS starch granules (Fig. 3 a–f), especially on the surface of CS. As the duration of APPJ pretreatment was prolonged, the DS increased, and the fluorescence intensity strengthened; these results were consistent with previous findings (Chen, He, & Huang, 2014). Cassava granules had a better reaction with OSA droplets as the duration of APPJ pretreatment was extended so that more  $-COO^-$  became available for  $MB^+$  staining. The fluorescence intensities of 10 min–OSA–starch, and 15 min–OSA–starch were higher than that of OSA–starch, 1 min–OSA–starch, 3 min–OSA–starch, 5 min–OSA–starch. The fluorescence intensities of OSA–starch, 1 min–OSA–starch, 3 min–OSA–starch and 5 min–OSA–starch granules were higher on the surface than the interior, indicating that the starch OS groups were mainly induced to the surface of the starch granules. However, in 10 min–OSA–starch and 15 min–OSA–starch, some starch granules showed the



**Fig 3.** CLSM optical sections of OSA-modified starches: (a) OSA-starch; (b) 1 min-OSA-CS; (c) 3 min-OSA-CS; (d) 5 min-OSA-CS; (e) 10 min-OSA-CS; and (f) 15 min-OSA-CS.

same fluorescence intensity inside and on the surface. This result illustrated more OS groups were induced to the internal starch granules as the duration of APPJ pretreatment was extended.

### 3.3.2. Thermal properties

The thermal properties of APPJ pretreatment and OSA-modified CS analyzed through DSC are reported in Table S1. The onset temperature ( $T_o$ ) of the APPJ-pretreated starch decreased as time increased compared with that of native CS. The result indicated that plasma pretreatment weakened the resistance of cassava granules to rupturing and swelling in aqueous media (Li, Shu, & Zhang, 2011). The enthalpy ( $\Delta H$ ) of CS decreased from  $14.93 \pm 0.14$  J/g to  $13.27 \pm 0.11$  J/g as the duration of APPJ pretreatment was prolonged from 0 min to 15 min. Similar results were also observed in nitrogen or helium glow-plasma-treated potato starch (Zhang, Chen, & Li, 2015). OSA modification decreased the onset temperature and  $\Delta H$  of native and pretreated CS.  $\Delta H$  indicates the degree to which starch molecules lose their double-helix structures (Wang et al., 2010). These results suggested that less energy was needed to initiate starch gelatinization, demonstrating that the crystalline region of CS was affected by esterification. The onset temperature and  $\Delta H$  decrease because the introduction of the hydrophobic alkenyl groups of OSA weakens internal hydrogen bonds and enhances structural flexibility; as a result, starch swells at a low temperature, and  $\Delta H$  gradually decreases (Lawal, 2004). Similarly,  $\Delta H$  of corn starch and potato starch decreased after modification with OSA (Tong, Deng, & Sun, 2019; Wang et al., 2019).

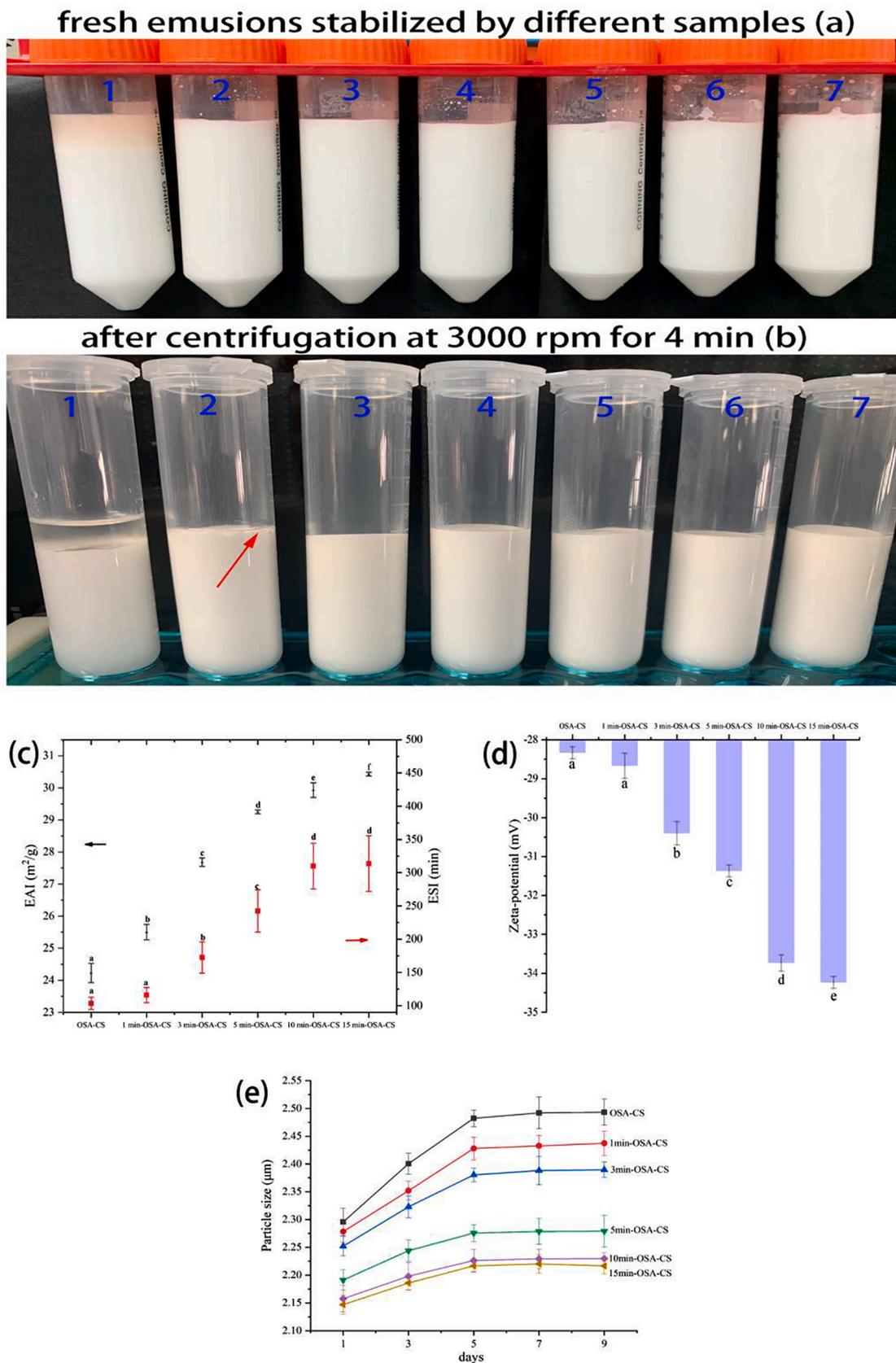
### 3.3.3. Visual assessment of starch-based emulsion stability

Fig. 4a showed the visual appearance of fresh emulsions stabilized by different starch samples. Emulsion stabilized by native CS showed the severest oiling-off at the top phase of the fresh emulsion. Owing to the hydrophilicity of native CS, most CS remained in the aqueous phase instead of being absorbed at the oil-water interface, which caused a limitation of their ability to stabilize the emulsions (Tong, Deng, & Sun, 2019). This result indicated native CS was not an excellent emulsifier to stabilize the emulsion. In the case of fresh emulsions stabilized by modified CS, no obvious stratification could be observed. The emulsion capacity of CS was enhanced after OSA modification; this result can be

attributed to the increase in DS which caused an increase in hydrophobicity. Because of the similar phenomenon in the emulsions stabilized by modified CS, centrifugation for 4 min at 3000 rpm was used to accelerate emulsion layering to investigate the physical stability of modified CS-based emulsions. As shown in Fig. 4b, the physical stability of the emulsions was closely related to the DS of OSA modified CS. Native CS exhibited an obvious separated oil layer at the top of the emulsion and a small amount of oil layer could also be seen in emulsion stabilized by OSA-CS. However, as the DS of OSA CS increased, no free oil was observed at top of emulsions stabilized by APPJ-OSA-CS under centrifugation, indicating that higher DS of OSA CS could prevent the oil phase from separating from the emulsion. All these results confirmed that OSA modified CS with higher DS can form a more homogenous layer of modified starch granules at the oil-water interface, thus obtaining better emulsifying capacity.

### 3.3.4. Emulsification performance

The emulsifying properties of OSA-CS and APPJ-OSA-CS are presented in Fig. 4c and Fig. 4d. EAI is often used to evaluate the emulsifying capacity. In this study, EAI increased from  $24.23 \pm 0.30$  m<sup>2</sup>/g to  $30.43 \pm 0.06$  m<sup>2</sup>/g as the DS of OSA CS increased. After OSA groups were introduced to CS, the starch granules became amphiphilic macromolecules, which cause OSA-modified starch to have a surface activity (Wang, Fu, & Tang, 2017). Similar results could be observed in ESI. ESI is utilized to evaluate the stability of emulsions. In this study, ESI increased from  $103.60 \pm 9.16$  min to  $313.59 \pm 41.85$  min as the DS of OSA-modified CS increased. APPJ-OSA-CS exhibited higher ESI value in contrast to OSA-CS. However, there was no significant difference between 10 min-OSA-CS and 15 min-OSA-CS. This result indicated the emulsion stability of OSA-modified CS was enhanced as DS increased. Steric hindrance may be an important factor affecting the stability of emulsions, and a densely packed layer is formed by OSA-modified starch at the oil-water interface, resulting in an increase of the steric hindrance of emulsion particles (Matos, Marefati, & Barrero, 2020). Electrostatic interaction is also a factor used to maintain the stability of emulsions. The presence of carboxyl groups in OSA favors the formation of a negatively charged emulsion (Lin, Liang, & Zhong, 2018a). As shown in Fig. 4d, the absolute zeta potential increased as the DS of OSA-modified



**Fig 4.** Visual appearance of emulsions prepared by different starch samples before (a) and after (b) centrifugation at 3000 rpm for 4 min. 1, Emulsion prepared with native CS; 2, emulsion prepared with OSA–CS; 3, emulsion prepared with 1 min–OSA–CS; 4, emulsion prepared with 3 min–OSA–CS; 5, emulsion prepared with 5 min–OSA–CS; 6, emulsion prepared with 10 min–OSA–CS; and 7, emulsion prepared with 15 min–OSA–CS. Emulsifying properties of OSA–CS and APPJ–OSA–CS (c, d) and changes in the particle size of emulsions prepared from modified starch within 9 days of storage (e).

CS increased. Compared with OSA-CS, the absolute value of zeta potential of APPJ-CS was higher, and 15 min-OSA-CS was the highest. The result indicated that APPJ-OSA-CS stabilized emulsion enhanced the electrostatic interaction between molecules, thus producing a more stable emulsion. The higher the DS, the greater the number of carboxyl groups at the oil-water surface of OSA-modified CS; consequently, the surface charge density is high (Lin, Liang, & Zhong, 2018b).

The storage stability of emulsion prepared by OSA-modified CS are presented in Fig. 4e. In the first 5 days, all emulsion samples exhibited an increase in the particle sizes and kept stable in the following 4 days. As the DS of CS increased, the particle sizes of starch emulsions decreased and the emulsion prepared by 15 min-OSA-starch showed the lowest particle sizes in contrast to other emulsion samples. With the increase of DS, the emulsified oil droplets in the emulsion were gathered together closely, which was conducive to the formation of a network structure based on droplets and enhances its stability against coalescence. A similar result was reported in OSA-modified rice starch, which showed that emulsions prepared by OSA modified rice starch with the DS of 0.026 had the lowest particle sizes after 10 day storage compared with that of emulsions prepared by rice starch with the DS of 0.021 and 0.024 (Li et al., 2018). Moreover, the emulsion prepared with 15 min-OSA-starch showed the smallest increase in particle size. This result indicated that this modified CS could be used as an excellent stabilizer for stabilizing emulsions.

### 3.4. Discussion on the mechanism of APPJ pretreatment to facilitate starch modification with OSA

Fig. 5 showed schematic diagram of plasma pretreatment to enhance the reaction efficiency between starch and OSA. The APPJ treated water contains a cocktail of active species, such as  $H_2O_2$ , nitrite, nitrite ions, nitric oxide and peroxyxynitrate (Liao et al., 2020). These active species could etch the surface of starch granules, causing different degrees of fragmentation on the surface of the starch granules (Fig. 2 and Fig. 5a), which is the main reason for the change in starch morphology. In addition, during APPJ pretreatment, the crystallinity and  $\Delta H$  decreased (Fig. 1c and Table S1), which indicated the loss of double-helix structure of starch (Fig. 5a). These structural disruptions increased the surface area for the reaction between starch and OSA, facilitating the penetration of OSA into the interior of starch granules to reach its molecular

chains (Fig. 3 and Fig. 5b). Consequently, the esterification between starch and OSA was enhanced. In addition to the etching effect, plasma treatment could introduce more hydrophilic groups such as hydroxyl groups to the starch (Fig. 1b). Based on this, we also proposed another potential mechanism. During the reaction, the five-membered ring of OSA was opened to form two hydrophilic groups (carboxyl groups) and one hydrophobic group, and one of the carboxyl groups underwent a dehydration condensation reaction with the hydroxyl group of the starch molecule to form an ester bond. The increase of hydrophilic groups (hydroxyl groups) of the starch molecule after plasma treatment could facilitate the condensation reaction, thus more OSA would be attached to the starch molecule (Fig. 5b).

## 4. Conclusion

APPJ could be an effective way to promote the modification of CS with OSA. The microstructure of starch granules was changed by the etching effect of APPJ. After CS was pretreated with APPJ, CS appeared as small clumps, and the surface of starch became rough. The crystallinity of CS decreased as the duration of APPJ pretreatment was prolonged. APPJ pretreatment could also introduce more hydrophilic groups such as hydroxyl groups to the starch. These structural changes promoted the OSA modification of CS. The DS and RE of OSA-modified CS increased as the APPJ pretreatment time was extended. CLSM showed that more OS groups were induced to the internal starch granules as the duration of APPJ pretreatment was extended. The onset temperature and  $\Delta H$  decreased after APPJ pretreatment and the OSA modification of starch samples. APPJ-OSA-CS also presented better emulsification performance in contrast to the control. All these results demonstrated that APPJ could be used as a novel way to assist the OSA modification of starch and provided useful information for utilization of OSA starch in the development of food grade emulsifier.

### CRediT authorship contribution statement

**Shengyang Ji:** Conceptualization, Investigation, Methodology, Writing - original draft. **Tao Xu:** Investigation, Writing - review & editing. **Weisu Huang:** Writing - review & editing. **Sunan Gao:** Writing - review & editing. **Yongheng Zhong:** Formal analysis. **Xuan Yang:** Formal analysis. **Mohamed Ahmed Hassan:** Formal analysis. **Baiyi Lu:**

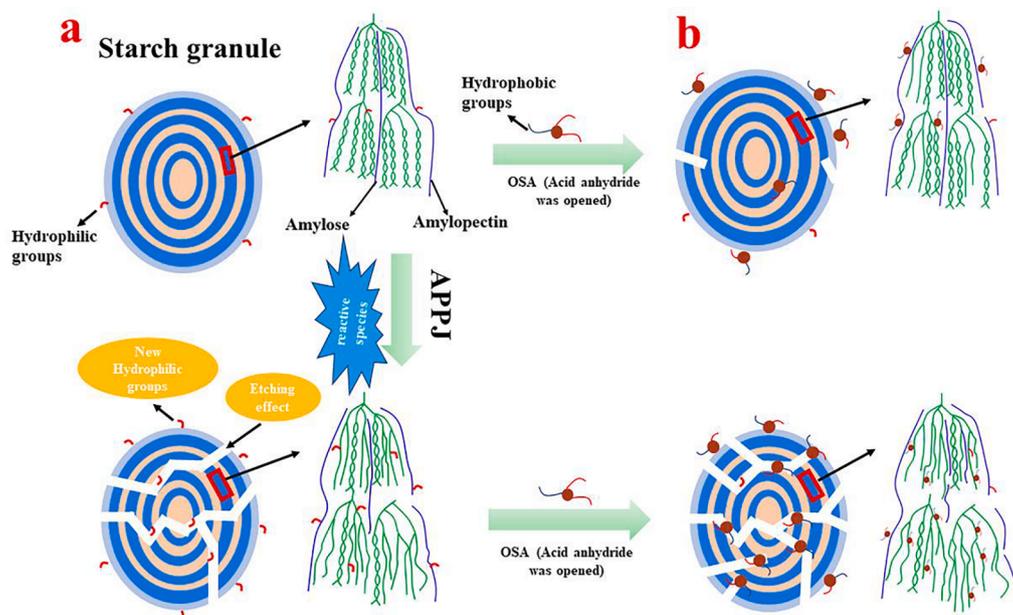


Fig. 5. Schematic diagram of plasma pretreatment to enhance the reaction efficiency between starch and OSA.

Conceptualization, Supervision, Project administration, Funding acquisition.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2021.130922>.

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