Modification and Characterization of Waxy and Native Tapioca Starches for Stabilizing Resveratrol Pickering Emulsion

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Abstract. Tapioca starch is a promising biopolymer for use as a solid particle stabilizer in Pickering emulsions. This study aimed to enhance the emulsifying performance of native (NS) and waxy (WS) tapioca starches through antisolvent precipitation followed by esterification with 3% (w/v) octenyl succinic anhydride (OSA). Structural and functional changes were characterized by SEM, XRD, DSC, FTIR, contact angle measurements, and degree of substitution analysis. The modifications transformed the starch morphology from smooth granules into rough, aggregated particles and reduced crystallinity, gelatinization enthalpy, and the intensity of characteristic functional groups. Both starch types exhibited similar structural changes; however, the modified native starch (NSp-OSA) showed higher hydrophobicity (contact angle: 103.73 ± 0.50°) than the modified waxy starch (WSp-OSA, 84.83 ± 0.60°), resulting in improved emulsion stability. Emulsion screening using jojoba oil identified optimal starch-to-oil ratios of 2.0:1.2 g/g for NSp-OSA and 1.0:1.2 g/g for WSp-OSA. These conditions were then applied for encapsulating resveratrol (RES). Due to the poor solubility of RES in oil, a ternary solvent system comprising of PEG 400, jojoba oil, and olive oil (0.1:0.5:0.5:0.5 g/g) was formulated to enhance solubility and emulsion uniformity. The NSp-OSA formulation produced more stable RES-loaded emulsions than WSp-OSA. This study demonstrates the potential of modified tapioca starches as bio-based stabilizers for emulsion-based delivery systems.

1 Introduction

Pickering emulsions, stabilized by solid particles instead of conventional surfactants, have gained increasing attention for applications in the food, pharmaceutical, and cosmetic industries due to their enhanced stability and biocompatibility [1]. Starch, a biodegradable and renewable biopolymer, is particularly attractive as an eco-friendly stabilizer [2]. Tapioca starch is abundant, inexpensive, and easily modified; however, native starch granules usually lack the amphiphilic properties required for effective stabilization of oil-in-water (O/W) emulsions [3]. To improve their emulsifying ability, several strategies such as particle size reduction and surface hydrophobization have been explored. Among these, esterification with octenyl succinic anhydride (OSA) is a widely used method to increase starch surface activity and promote interfacial adsorption in emulsion systems [4, 5].

Resveratrol (RES), a naturally occurring polyphenolic compound, has attracted considerable attention due to its antioxidant, anti-inflammatory, and anticancer properties. Despite its therapeutic potential, the practical use of RES in functional food, cosmetic, and pharmaceutical formulations is limited by its poor water solubility, low chemical stability, and limited bioavailability. Emulsion-based delivery systems offer an approach to enhance the solubility and stability of RES in aqueous formulations [6]. Successful encapsulation requires a formulation that can dissolve RES efficiently in the oil phase while maintains emulsion stability during storage. Pickering emulsions stabilized by modified starch particles represent a promising and sustainable strategy to achieving these goals using food-grade, bio-based materials [7, 8].

This study focuses on improving the emulsifying properties of native (NS) and waxy (WS) tapioca starches for stabilizing RES-loaded Pickering emulsions. Antisolvent precipitation using ethanol was employed to reduce particle size, followed by esterification with 3% (w/v) OSA to enhance hydrophobicity. The modified starches (NSp-OSA and WSp-OSA) were characterized in terms of morphology, crystallinity, thermal behavior, chemical properties, and wettability using SEM, XRD, DSC, FTIR, degree of substitution, and contact angle analyses. Their performance as stabilizers was evaluated through emulsion screening with jojoba oil and subsequent RES encapsulation using a ternary oil mixture designed to enhance RES solubility. This work provides comparative insights into how structural and surface property differences between modified native and waxy tapioca starches influence their ability to stabilize RES-loaded Pickering emulsions.

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2 Materials and Methods

2.1 Materials

Waxy and native tapioca starches were supplied by Thai Wah Public Co., Ltd. (Thailand). Ethanol was obtained from the Liquor Distillery Organization (Thailand), while octenyl succinic anhydride (OSA) was sourced from Tokyo Chemical Industry Co., Ltd. (Japan). Hydrochloric acid was purchased from RCI Labscan Co., Ltd. (Thailand), and sodium hydroxide from Supelco Inc. Cosmetic-grade *trans*-resveratrol, jojoba oil, and olive oil were acquired from Chanjao Longevity Co., Ltd. (Thailand). Polyethylene glycol 400 (PEG 400, cosmetic grade) was provided by S. Tong Chemicals (1985) Co., Ltd. Unless otherwise specified, all chemicals used were of analytical grade.

2.2 Preparation of nanoprecipitated starch

Two types of tapioca starch—native starch (NS) and waxy starch (WS)—were subjected to particle size reduction via antisolvent precipitation using ethanol, also known as ethanol nanoprecipitation, following a method adapted from [9]. A 1% (w/v) starch suspension was prepared by dispersing 8.00 g of starch in 800 mL of deionized (DI) water and stirring continuously at 200 rpm and 100°C for 30 min to achieve complete gelatinization. Once a homogeneous starch solution was obtained, the mixture was allowed to cool to room temperature (~30°C) while stirring continued. Ethanol (160 mL) was then added dropwise at a constant rate under continuous stirring. The mixture was stirred for an additional 8 h at ambient temperature. Subsequently, it was refrigerated at approximately 4°C for 15 h to promote starch precipitation. The resulting precipitate was collected and freeze-dried for 48 h, or until completely dry, yielding native starch precipitate (NSp) and waxy starch precipitate (WSp).

2.3 OSA modification of nanoprecipitated starch

NSp and WSp were each weighed at 5.00 g and dispersed in 95.00 mL of DI water. The suspension was stirred at 200 rpm at 35°C, and the pH was adjusted to 8.0–8.5 using a 3% (w/v) sodium hydroxide solution. Octenyl succinic anhydride (OSA) was then added at a concentration of 3% (w/v), based on the dry weight of starch. The reaction was carried out for 3 h under continuous stirring. After the reaction, the pH of the mixture was adjusted to 6.5 using 0.5 M hydrochloric acid solution. The modified starch was recovered by centrifugation at 5,000 rpm for 3 min. The supernatant was discarded, and the precipitate was washed with DI water and centrifuged again under the same conditions. This washing step was repeated twice. The resulting precipitate was then washed twice with 97% (v/v) ethanol and dried in an oven at 40°C for 24 h. The final products obtained were designated as NSp-OSA and WSp-OSA.

2.4 Characterization of starch

2.4.1 SEM analysis

The morphology of tapioca starch granules before and after OSA modification was examined using a scanning electron microscope or SEM (JEOL JSM-7610FPlus) at 3000× magnification to evaluate granule shape and surface characteristics. Samples were mounted on SEM stubs using carbon adhesive tape and directly introduced into the chamber for imaging [10].

2.4.2 X-ray diffraction

The crystalline structure of native and modified starches was analyzed via X-ray diffraction (XRD) using a D2 PHASER diffractometer (Bruker, Germany) equipped with Cu-Kα radiation (λ = 1.5406 Å), operating at 30 kV and 10 mA. Scans were recorded over a 2θ range of 5° to 60°, with a step size of 0.02° and a dwell time of 0.6 s per step. The relative crystallinity was calculated using Equation (1) [11]:

$$\text{Crystallinity} = (\text{Area under the peaks}) \times 100 / (\text{Total curve area}) \quad (1)$$

2.4.3 Thermal properties

Thermal transitions, particularly gelatinization behavior, were assessed by differential scanning calorimetry (DSC). Prior to analysis, starch samples were dried to a moisture content of approximately 7.7%. Around 10.623 mg of starch was suspended in deionized water at 30% (w/w) and loaded into a 60 µL stainless steel pan (CODE03190029) sealed with a volatile-resistant lid and tygon O-rings. Samples were equilibrated overnight at room temperature. DSC analysis was conducted from 0°C to 120°C at a heating rate of 10°C/min under a nitrogen atmosphere (flow rate: 20 mL/min) [11, 12]. The onset (To), peak (Tp), and conclusion (Tc) temperatures, along with gelatinization enthalpy (ΔH), were analyzed.

2.4.4 FTIR

Functional groups of the starches were characterized using a FT-IR spectrometer (PerkinElmer Spectrum 100, USA). The samples were mixed with potassium bromide (KBr) and pressed into translucent pellets using a manual hydraulic press at 5 N/m^2 for 3 min. FT-IR spectra were collected in the 450–4000 cm^{-1} range with a resolution of 4 cm^{-1}, averaging eight scans per sample [13].

2.4.5 Contact angle

Surface wettability of the starches before and after OSA modification was evaluated using water contact angle measurements. Samples were compressed into flat pellets using a manual hydraulic press at 5 N/m^2 for 3 min. A 5 µL droplet of deionized water was dispensed onto the sample surface at a controlled rate of 0.5 µL/s using a microsyringe. Measurements were conducted using an optical contact angle system (OCA15 Plus, DataPhysics Instruments GmbH, Filderstadt, Germany) with a 6×

parfocal zoom lens [14]. Each sample was analyzed in triplicate for reproducibility.

2.4.6 Degree of substitution

The degree of substitution (DS) of OSA-modified starches was determined using a titration method adapted from [15]. In brief, 1.00 g of modified starch was dispersed in 25.0 mL of 2.5 M HCl in isopropanol and stirred at 300 rpm for 10 min at room temperature. Subsequently, 20.0 mL of 90% (v/v) aqueous isopropanol was added, and the slurry was stirred continuously for 30 min. The mixture was then centrifuged at 300 rpm for 30 min. The supernatant was titrated with 0.1 M NaOH using phenolphthalein as the indicator. The DS was calculated using Equation (2):

$$DS = [0.162 \times (A \times M) / W]/[1 - (0.210 \times (A \times M) / W)] \quad (2)$$

where A is the volume of NaOH (mL), M is the molarity of NaOH (mol/L), and W is the weight of starch sample (g).

2.5 Pickering emulsion formulation and evaluation

The preparation of Pickering emulsions was adapted from the method described in [16]. NSp-OSA and WSp-OSA were dispersed in DI water according to the compositions listed in Table 1. The starch suspensions were stirred at 200 rpm and 35°C for 10 min to ensure homogeneity of the aqueous phase. Jojoba oil was then gradually added dropwise to the suspension while homogenizing with a high-speed homogenizer at 15,000 rpm. After the complete addition of oil, homogenization was continued for an additional 3 min. The resulting emulsions were transferred into glass vials for stability testing at room temperature (~30°C). The physical appearance of the emulsions was monitored at time intervals of 5 min, 30 min, 1 h, and 24 h. Once the optimal Pickering emulsion formulation for jojoba oil was identified, it was used for resveratrol (RES) encapsulation. The final formulation consisted of 0.5% (w/w) jojoba oil, 0.1% (w/w) RES, 0.5% (w/w) polyethylene glycol 400 (PEG 400), and 0.5% (w/w) olive oil, with DI water added to adjust the total formulation to 100% (w/w).

Table 1. Formatting sections, subsections and subsubsections.

NSp-OSA or WSp-OSA (g)	Jojoba oil (g)	DI water (g)
0.05	1.20	98.75
0.10		98.70
0.20		98.60
1.00		97.80
2.00		96.00

3 Results and discussion

3.1 SEM analysis

The morphological characteristics of NS, WS, NSp, WSp, NSp-OSA, and WSp-OSA were examined using SEM, as illustrated in Figure 1. Native starches (NS, WS) exhibited smooth, spherical granules (~15–16 µm). Ethanol precipitation (NSp, WSp) caused partial granule disruption and increased surface roughness. After OSA modification (NSp-OSA, WSp-OSA), granules lost their spherical shape, appearing as rough, stone-like aggregates. Both starch types showed similar morphological changes throughout the process.

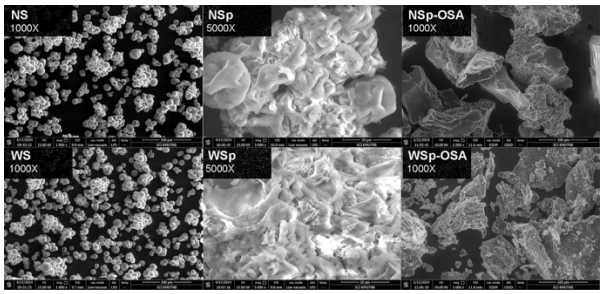

Fig. 1. SEM images of NSp, WSp, NSp-OSA, and WSp-OSA.

3.2 X-ray diffraction

Tapioca starch possesses a semicrystalline structure composed primarily of amylose and amylopectin. Native starches (NS and WS) exhibited distinct XRD peaks at 15°, 17°, and 23°, reflecting their high crystallinity (Figure 2). In contrast, ethanol-precipitated samples (NSp and WSp) showed broader peaks, indicating partial disruption of the crystalline regions. Following OSA modification, NSp-OSA and WSp-OSA displayed a single broad peak around 20°, suggesting significant structural rearrangement and a transition toward a more amorphous state due to esterification. Correspondingly, crystallinity decreased from 22.4% (NS) to 7.2% (NSp-OSA) and from 22.8% (WS) to 7.9% (WSp-OSA).

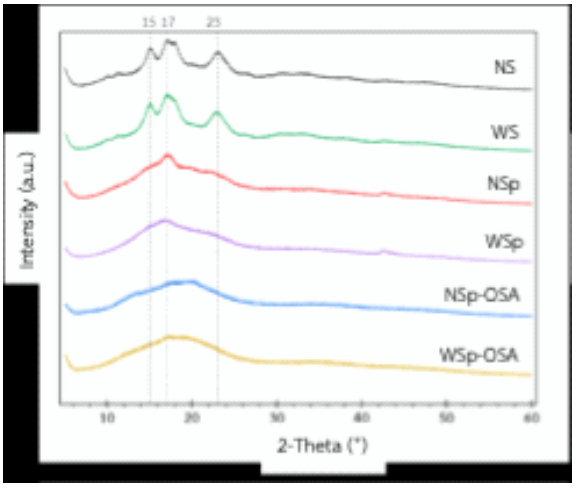

Fig. 2. XRD patterns of NS, WS, NSp, WSp, NSp-OSA, and WSp-OSA.

3.3 Thermal properties

Figure 3 and Table 2 show the DSC thermograms and thermal properties of NS, WS, NSp-OSA, and WSp-OSA, highlighting the impact of modification on starch gelatinization. Native starches (NS and WS) exhibited onset temperatures (To) of 62.16 ± 0.33°C and 66.38 ± 0.12°C, with peak temperatures (Tp) at 69.09 ± 0.12°C and 72.34 ± 0.23°C, respectively. In contrast, OSA-modified starches (NSp-OSA and WSp-OSA) showed significantly lower To values of 23.14 ± 1.14°C and 34.85 ± 0.09°C, along with a marked reduction in gelatinization enthalpy (ΔH)—from 16.16 ± 0.36 J/g to 0.18 ± 0.07 J/g for NSp-OSA, and from 17.43 ± 0.03 J/g to 0.96 ± 0.00 J/g for WSp-OSA.

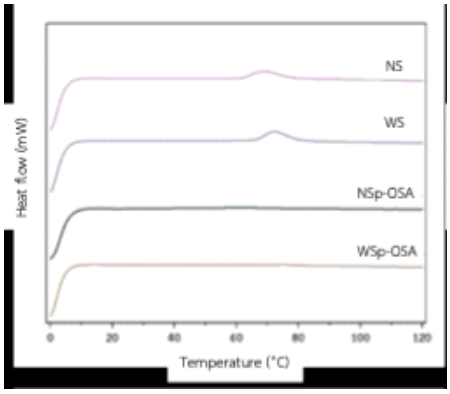

Fig. 3. DSC thermograms of NS, WS, NSp-OSA, and WSp-OSA.

These thermal changes indicate disruption of crystalline regions due to ethanol precipitation and OSA esterification, leading to reduced particle size, weakened hydrogen bonding, and delayed retrogradation. The lower gelatinization temperature enables the formation of gel-like surface layers under mild heating, enhancing particle surface activity and promoting more effective adsorption at the oil–water interface, thereby improving Pickering emulsion stability.

Table 2. Thermal properties of NS, WS, NSp-OSA, and WSp-OSA.

Sample	To (°C)	Tp (°C)	Tc (°C)	Enthalpy (ΔH, J/g)
NS	62.16 ± 0.33	69.09 ± 0.12	78.62 ± 0.33	16.16 ± 0.36
WS	66.38 ± 0.12	72.34 ± 0.23	80.43 ± 0.16	17.43 ± 0.03
NSp-OSA	23.14 ± 1.14	30.37 ± 0.51	34.93 ± 1.28	0.18 ± 0.07
WSp-OSA	34.85 ± 0.09	62.06 ± 0.08	68.60 ± 0.41	0.96 ± 0.00

3.4 FTIR

FT-IR analysis (Figure 4) confirmed the successful functionalization of tapioca starch with OSA. Native starches (NS and WS) exhibited characteristic absorption bands at ~3400 cm^{-1} (O–H stretching), 2920 cm^{-1} (C–H stretching), 1645 cm^{-1} (O–H bending of absorbed water), and 1018 cm^{-1} (C–O–C stretching of the glycosidic lickage). The ethanol-precipitated starches (NSp and WSp) showed broader O–H stretching bands, indicating increased hydrophilicity due to partial disruption of hydrogen bonding. In contrast, the OSA-modified starches (NSp-OSA and WSp-OSA) displayed new absorption peaks at 1720 cm^{-1} (C=O stretching of ester groups) and 1570 cm^{-1} (asymmetric stretching of $RCOO^{-}$), confirming successful esterification. However, these peaks were relatively weak, suggesting a low degree of substitution and indicating that complementary analytical methods may be required for further varification.

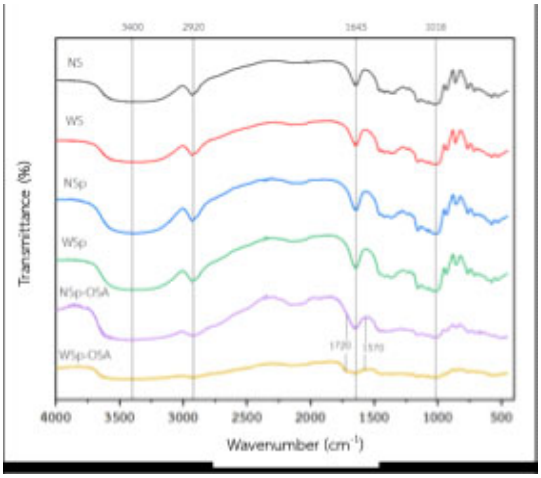

Fig. 4. FT-IR spectra of NS, WS, NSp, WSp, NSp-OSA, and WSp-OSA.

3.5 Degree of substitution and contact angle

The degree of substitution (DS) was evaluated to confirm the successful grafting of OSA onto starch molecules. The DS values for NSp-OSA and WSp-OSA were 0.032 ± 0.005 and 0.039 ± 0.009, respectively. As shown in Figure 5, native starches (NS and WS) exhibited water contact angles of 48.03 ± 1.28° and 52.63 ± 0.32°, respectively. These values increased slightly after ethanol precipitation to 65.00 ± 0.76° for NSp and 59.50 ± 0.36° for WSp. Following OSA modification, the contact angles increased significantly to 103.73 ± 0.50° for NSp-OSA and 84.83 ± 0.60° for WSp-OSA. This marked increase in hydrophobicity confirms the successful substitution of hydrophilic hydroxyl groups with lipophilic octenyl groups via esterification, in agreement with both FTIR and DS results.

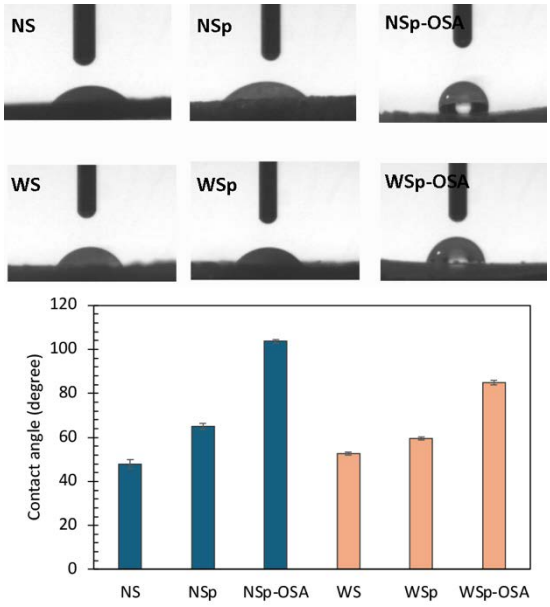

Fig. 5. Contact angle of NS, WS, NSp, WSp, NSp-OSA, and WSp-OSA.

3.6 Screening formulation of Pickering emulsion using NSp-OSA and WSp-OSA

To evaluate the emulsifying performance of modified tapioca starches, Pickering emulsions were prepared using 3% w/v NSp-OSA or WSp-OSA with varying starch-to-oil ratios, keeping the oil phase (jojoba oil) constant. Emulsion stability was assessed over 24 h at room temperature, as shown in Figure 6.

At lower starch-to-oil ratios (e.g., 0.05:1.2 g/g), emulsions appeared milky and homogeneous with minimal phase separation initially. Over time, both NSp-OSA-0.05 and WSp-OSA-0.05 showed slight creaming and increased clarity. Similar behavior was observed at 0.1:1.2 and 0.2:1.2 g/g, with gradual cream layer formation and minor starch sedimentation after 24 h.

At higher ratios (1.0:1.2 and 2.0:1.2 g/g), NSp-OSA-1.0 and NSp-OSA-2.0 emulsions remained stable with minimal separation, while WSp-OSA-1.0 showed slight sedimentation and WSp-OSA-2.0 displayed evident creaming and reduced stability.

In summary, NSp-OSA-2.0 and WSp-OSA-1.0 yielded the most stable emulsions. The greater contact angle of NSp-OSA (>90°) compared to WSp-OSA (~90°) enhanced its stabilizing ability. Excessive starch (e.g., WSp-OSA-2.0) resulted in foaming and instability.

Based on the results, the optimal Pickering emulsion formulations for resveratrol encapsulation were NSp-OSA-2.0 and WSp-OSA-1.0, which exhibited the most stable physical properties. These emulsions were selected for further use in encapsulating resveratrol.

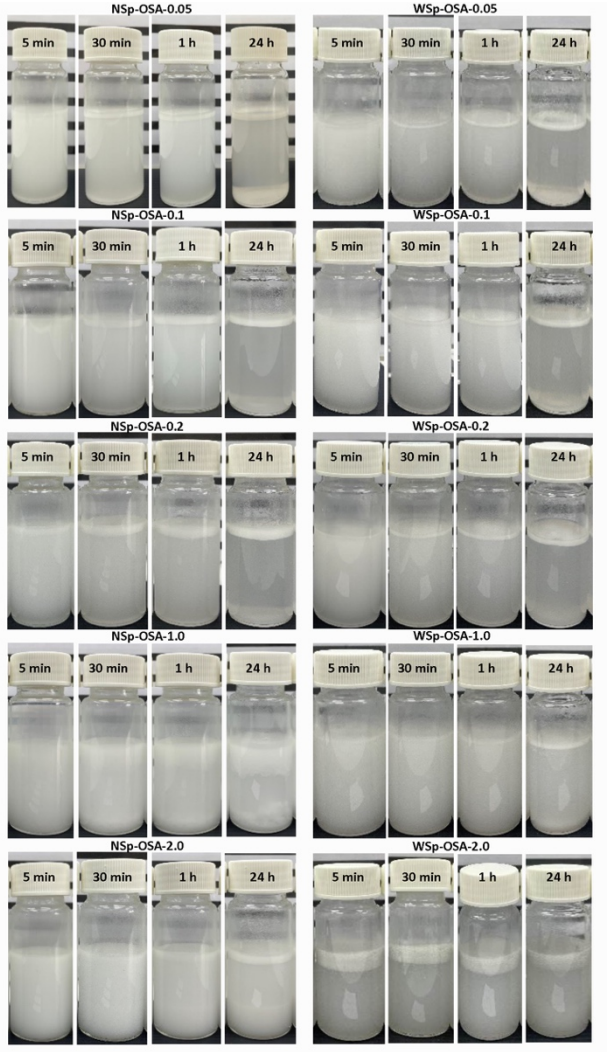

Fig. 6. Pickering emulsions prepared with NSp-OSA and WSp-OSA at various ratios and leave at room temperature for 24 h.

3.7 Solubility test and Pickering emulsion of RES

Prior to encapsulation in the Pickering emulsion, the solubility of RES was evaluated, as shown in Figure 7. In the first trial, using a 0.1:0.5 g/g ratio of RES to DI water, RES did not dissolve. Similarly, in the second trial, with a 0.1:0.5 g/g ratio of RES to jojoba oil (JO), RES remained undissolved. However, in the third trial, where a 0.1:0.5 g/g ratio of RES to PEG 400 (PEG) was tested, the RES dissolved completely, forming a clear solution.

To improve the solubility of 0.1 g of RES in jojoba oil (JO), a mixture of JO and PEG at a weight ratio of 0.5:0.5 was initially tested; however, slight phase separation was observed. Subsequently, the addition of olive oil (OO), forming a JO:PEG:OO mixture at a weight ratio of 0.5:0.5:0.5, resulted in enhanced solubility and a more homogeneous solution. Therefore, the formulation with a RES:PEG:JO:OO ratio of 0.1:0.5:0.5:0.5 g was selected for resveratrol encapsulation in the Pickering emulsion.

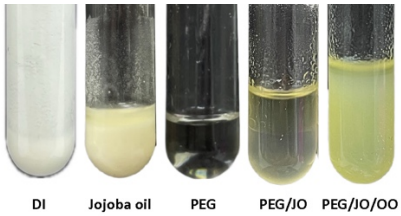

Fig. 7. Solubility of RES in DI water, JO, PEG400, and mixed solvents.

Pickering emulsion of RES using NSp-OSA and WSp-OSA were prepared using 1.2 g of oil phase comprising RES, PEG, JO, and OO at a weight ratio of 0.1:0.5:0.5:0.5. The emulsions were stored at room temperature (~30°C) for 24 h, as shown in Figure 8. The results indicate that the stability of the RES Pickering emulsions was significantly enhanced compared to those formulated with NSp-OSA-1.0 and WSp-OSA-2.0 in the presence of JO as the single oil component.

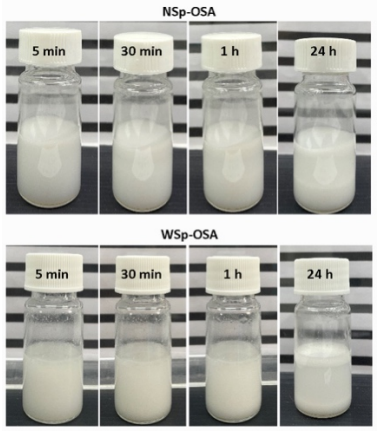

Fig. 8. Pickering emulsions of RES prepared using NSp-OSA-0.1 and WSp-OSA-2.0 with and oil phase comprising RES, PEG, JO, and OO at a weight ratio of 0.1:0.5:0.5:0.5, observed over a 24 h period at room temperature (~30 °C).

4 Conclusion

This study investigated the modification of native (NS) and waxy tapioca (WS) starches through antisolvent precipitation to reduce particle size and esterification with 3% (w/v) octenyl succinic anhydride (OSA) to enhance particle hydrophobicity for use in Pickering emulsion formulations. The modifications altered the starch morphology from smooth, spherical granules to rough, stone-like aggregates. Crystallinity decreased after modification, resulting in lower gelatinization enthalpy. Both starch types exhibited similar trends in morphology, crystallinity, thermal behavior, and functional group changes; however, they differed markedly in hydrophobicity, as reflected by water contact angles of $103.73 \pm 0.50°$ for NSp-OSA and $84.83 \pm 0.60°$ for WSp-OSA. This difference in surface hydrophobicity significantly affected their ability to stabilize Pickering emulsions.

Screening with jojoba oil identified optimal formulations at starch-to-oil ratios of 2.0:1.2 g/g for NSp-OSA and 1.0:1.2 g/g for WSp-OSA. These conditions were applied for resveratrol (RES) encapsulation. Solubility testing indicated that RES had poor solubility in jojoba but dissolved well in PEG 400, while the addition of olive oil improved the homogeneity of the oil phase. Consequently, a mixed oil system (RES: PEG : JO : OO at 0.1:0.5:0.5:0.5 g/g) was employed for preparing RES-loaded Pickering emulsions. This formulation significantly improved emulsion stability, with NSp-OSA demonstrating superior performance compared to WSp-OSA.

Overall, this study provides valuable insights into the functional enhancement of tapioca starches and their potential as bio-based stabilizers for emulsion-based delivery systems and other value-added applications.

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Author contributions

Rapeeporn Wannarat.: Investigation and Methodology. **Anchana Pleanklay.**: Investigation and Methodology. **Suthida Boonsith.**: Conceptualization, Resources, and Validation. **Sukanya Thepwatee.**: Conceptualization, Data Curation, Formal Analysis, Graphic Preparation, Writing – Original Draft, Reviewing, and Editing, Funding Acquisition, Supervision, Project Administration, and Validation.

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