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Original Article

Evaluation of native and modified *Dioscorea hispida* starches for use in Pickering emulsion stabilisation

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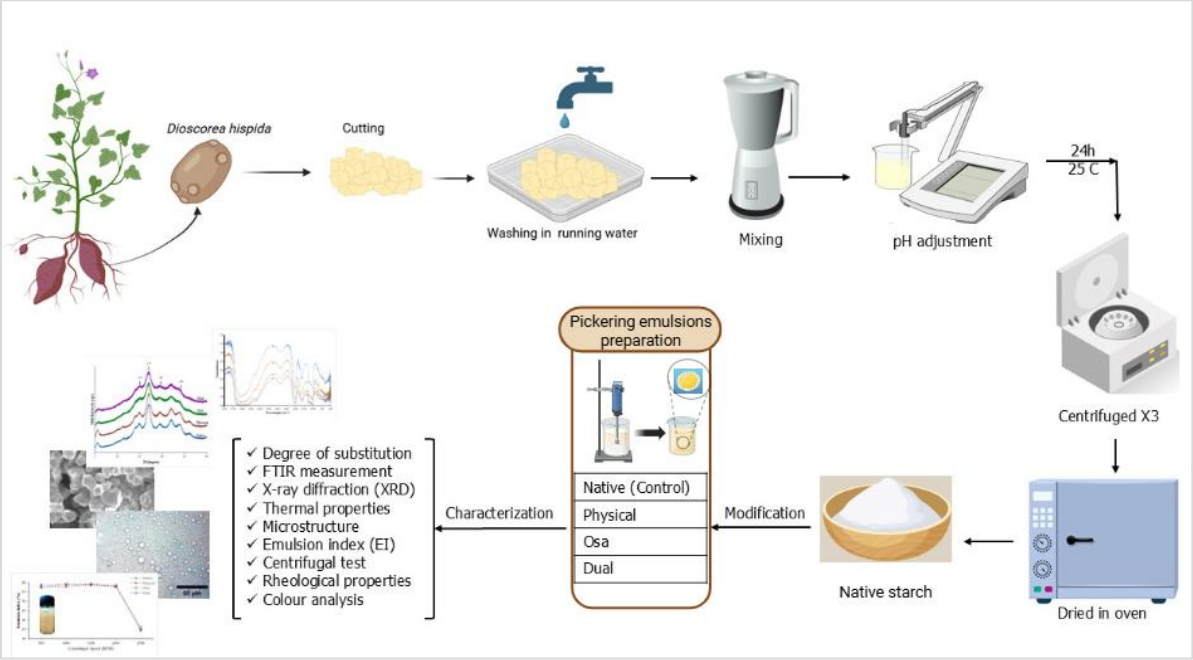
Abstract

Naturally abundance in the versatile functional properties has allowed starch to gain interest across industries like food, pharmaceuticals, and cosmetics. *Dioscorea hispida* Dennst. (*D. hispida),* a wild tuber, native to some regions, has been considered as potentially useful for industrial applications following its availability and unexplored attributes. This study explores the complementary effects of various modification methods, compromising heat (physical), octenyl succinic anhydride (OSA) esterification (chemical), and dual treatment, with *D. hispida* starch as the single stabiliser in Pickering emulsion system. Upon formulation as emulsifiers in the Pickering emulsions, the starches microstructure, creaming, stability upon centrifugation, rheological performance and colour were categorised. The molecular structure, crystallinity, morphology and thermal attributes of the starches were examined to assess their stability for emulsification. Modification of starches led to the increase of starch granules to 3.52 µm, from 3.04 µm. Lowest emulsion stability after four weeks was demonstrated by dual modification (78.26 %), associated with 23.84 µm of droplets' sizes. As indictment of their flow properties, all Pickering emulsion samples exhibited shear-thinning rheological behaviour. These findings implored that *D. hispida* starch is a promising substitution for traditional stabiliser, ensuring a green-label ingredient. Extensive research is recommended to evaluate its stability in the long run for further applications.

Keywords: Starch modification, *D. hispida*, Pickering emulsion, Synergistic effect

Graphical Abstract



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Introduction

Stabilising emulsion with solid particles instead of surfactant is known as Pickering emulsions, offers enhanced stability through permanent adsorption at the oil-water interface. The droplets coalescence is therefore avoided and ensuring greater stability than surfactant-based emulsions. The stabilisers chosen are vital, with biodegradable and biocompatible materials like starch and cellulose derivatives being useful material in biomedicine and food industries due to their natural origin and sustainable sources (Ramos et al., 2025). The proportion of the emulsions' droplet also varies based on the stabiliser type, with smaller droplet sizes demonstrating longer stability, with some able to remain stable for eight years (Bai et al., 2021). Combination of amylose, amylopectin, nanocrystals (orthorhombic and/ or hexagonal) including minerals and salts forming a micro- or submicron-sized particles known as starch (Ai and Jane, 2024). Existing as granules within the vegetative plant parts including grains, tubers, fruits and roots, it is constituted of two dominant macromolecules (amylose and amylopectin) (Abdul Hamid et al., 2020). These water-insoluble macromolecules are products of the linking of hydrogen bonds and hydrophobic bonds (He et al., 2024). Also divided into a range of sizes, starch granules can be identified as particularly small granules (0.3-2 μm), small granules (2-10 μm), medium granules (5-30 μm), and large granules (30-100 μm) (Abdul Hadi et al., 2020b).

The native starches have been used widely in daily life; however, their properties have some disadvantages, including an incapability to construct stable starch and gels, leading to deterioration of viscosity, and insolvability in low temperature water, hence limiting their usage in the industry. However, the starch functionality can be improved by modifying the native form of starches, which involves the use of techniques like chemical (esterification, oxidation, and etherification), physical (heat and milling), enzymatic, and genetic modification (Abdul Hadi et al., 2020b). Altered starch, either chemically or physically, to improve and develop desirable functional properties for a specific industry is called modified starch (Zhang et al., 2021b).

The most extensively investigated technique of starch modification is chemical modification by esterification or acylation. The example includes the alteration with octenyl succinic anhydride (OSA). OSA's esterified starch is normally exploited as an emulsifier (esterification agent) in food products, including beverages, salad dressings, or encapsulating agents. OSA alteration integrates hydrophobic groups onto starch molecules, with amphiphilic derivative, which acts as a stabiliser for emulsion (Zhang et al., 2021b). This also includes the fractional replacement of hydroxyl groups with hydrophobic substituents, thereby supplying an amphiphilic character and interfacial properties within the starch. The physicochemical attributes of the OSA-modified starches will differ based on the starch used as its crude material, the treatment circumstances used during the modification (including conditions if pre-treatments/ post-treatments were conducted), the range of derivatisation, and the dispersal of the newly introduced groups within the starch granules (Sharma et al., 2025). The U.S. Food and Drug Administration (FDA) has limited the OSA addition applied for modification maximum of 3% based on the weight of starch (Sharma et al., 2025).

Despite the extensive research on OSA-modified starch derived from countless conventional botanical sources including maize, potato, rice, and quinoa, little to no studies have explored the starch from underutilized or toxic tubers like *Dioscorea hispida* Dennst (*D. hispida),* which is commonly known as 'gadong'. *D. hispida* is methodically proven to possess therapeutic effects with the presence of bioactive components, like saponins and dioscorine (Subroto et al., 2024). This study introduces a novel approach by modifying the starch extracted from *D. hispida* for use as a Pickering emulsion as an emulsifier, thereby valorising an underutilised and toxic species into a functional and sustainable biomaterial.

Materials and Methods

Materials and chemicals

The wild *D. hispida* tubers were obtained from a rural forest located in Kuala Berang, Hulu Terengganu, Malaysia. The tubers were from white tuber cultivar, grown naturally in forest area during rainfall season. Sodium hydroxide (NaOH) pellets, hydrochloric acid (HCl), phenolphthalein solution, octenyl succinyl anhydride (OSA), and potassium bromide (KBr) were of analytical calibre.

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Starch preparation

Starch isolation

The starch was extracted from *D. hispida* tubers using the method by Hazrati et al. (2021). The tubers were washed, sliced, and ground with 1:1 (v/v) ratio in water. The paste was then mixed, and alkaline steeping was conducted by adding 0.25 M NaOH to reach pH 11, before being left to soak overnight. The paste was centrifuged (6,000 rpm, 10 minutes) at room temperature. The aqueous phase was then removed. The starch layer (bottom part) was washed by suspending it in distilled water and centrifuged. The washing step was carried out thrice. The starches were then dried in an oven at 60 ℃ overnight.

Preparation of partially gelatinised *D. hispida* starch

The native *D. hispida* starches (200 g, starch concentration 10 wt.%) were heated at 85 ℃ for 20 minutes with occasional manual disturbance. The samples were left to dry at 55 ℃ overnight, and ground before stored at room temperature.

Preparation of OSA and dual-modified *D. hispida* starch

Both native and partially gelatinised *D. hispida* starch were used for the OSA modification following a method by Lopez-Silva et al. (2022) with a slight modification. Native or partially gelatinised *D. hispida* starch samples were mixed in distilled water with a ratio of 1:7 under gentle stirring at 500 rpm. The pH was altered to 11 using 0.7 N NaOH. OSA (3.0%) from the total dry starch was added simultaneously with 0.7 N NaOH to sustain pH of 8.5 ± 0.1. The reaction was left to continue for 4 h at room temperature until the pH remained constant, as an indictment for complete reaction of added anhydride with the $OH^{(-)}$ groups of the starch structure. Then, alteration of the pH to 7.0 with 1.0 N HCl was conducted. The centrifugated modified starch solution was then washed and rinsed with ethanol, before dried at 40 ℃ until constant weight is achieved.

Preparation of *D. hispida* starch stabilised Pickering emulsion

Oil-in-water *D. hispida* starch Pickering emulsion was prepared with a formulation comprised of 20% (v/v) of medium-chain triglyceride oil (palm oil) as the dispersed phase and 80% (v/v) water as the continuous phase. The emulsion was stabilised with 200 mg *D. hispida* starch per mL of palm oil. The starch concentration was chosen based on findings that indicate 200 mg of starch is enough to form a stable Pickering emulsion system (Abdul Hadi et al., 2020a). The *D. hispida* starch was dispersed in water. After the addition of palm oil, the mixture was homogenised at 22,000 rpm for 1 minute using an Ultra-Turrax® rotor-stator type homogeniser (IKA T18 Digital, IKA®-Werke GmbH & Co., Germany). Four types of Pickering emulsion samples, namely native, physical, OSA and dual, were prepared from the native and modified *D. hispida* starches.

Characterisation of *D. hispida* starch granules

Degree of substitution of starches

The degree of substitution (DS) of OSA and dual-modified *D. hispida* starches was calculated using the titration technique by Marefati et al. (2017) with a peripheral amendment. The OSA and dual starch samples were diffused in distilled water at a 1:10 ratio. Approximately 25 mL of 0.5 M NaOH was added to the starch slurry with continuous stirring and shaken overnight at room temperature. Remaining alkali was titrated with 0.5 M HCl, with phenolphthalein as an indicator. The DS was determined from the % OSA substitution and calculated using the following formula described by Zainal Abiddin et al. (2015):

$$\text{OSA substitution (\%)} = \frac{(V_{blank} - V_{sample}) \times 0.1 \times M \times 100}{W} \quad (1)$$

where

W = dry weight of OSA sample

M = molarity of HCl solution

V_{blank} = volume of HCl required for blank titration

V_{sample} = volume of HCl required to titrate the sample

$$DS = \frac{162 \times \text{OSA substitution (\%)}}{21000 - (209 \times \text{OSA substitution (\%)})} \quad (2)$$

where

162 = molecular weight of glucose unit

21000 = (100) (molecular weight of OSA group) - molecular weight of H_2.

FTIR measurement

A Tensor 37 FTIR spectrometer (IS10, Thermo Scientific Inc., USA); (4,000-400 cm^{-1} range; 4 cm^{-1} resolution) was employed to characterize *D. hispida*

starches. The samples of *D. hispida* starches were mixed thoroughly with 250 mg of KBr, and then 150 g of the blend was pressed into pellet by applying 115 kN (8700 bar) for 1 minute. Following baseline correction, the recorded transmittance spectra were converted to absorbance spectra for further analysis.

X-ray diffraction (XRD)

The crystalline structure of the starches was analysed using Small Angle X-Ray Scattering (SAXS) (Rigaku Smartlab SE, Japan), recorded over a 2θ range of 5˚ to 30˚ at ambient temperature.

Morphology

The morphology of *D. hispida* starch granules was monitored using a scanning electron microscope (SEM; JSM-6610LV, JEOL Ltd., Japan). The samples were fixed onto aluminium stubs with 20 nm palladium/gold coating prior to imaging. The micrographs were obtained at an accelerated voltage of 5 kV at 1,000 and 5,000 magnifications.

Thermal properties

The thermal behaviour of native and modified *D. hispida* starches was characterised using a differential scanning calorimeter (DSC8000, Perkin Elmer, USA). The starch dispersions (1:5 ratio) in the buffer were heated in a range of 10℃ to 120℃ using a scanning speed of 10℃ min^{-1}. The temperature at the onset of gelatinisation (TO), peak gelatinisation temperature (TP), final gelatinisation temperature (TC), and gelatinisation enthalpy (ΔH) were verified.

Microstructure of Pickering emulsion

The microstructure of all *D. hispida* Pickering emulsions was examined using light microscopy (Olympus BX50, Japan). The Pickering emulsions were mixed in a phosphate buffer with a 1:9 ratios before mixed upon observation. Four drops of each emulsion were placed on the slide without a cover slip. The images of the emulsion droplets were captured under objective magnifications of 4x and 10x using IC Capture software.

Creaming index

Approximately 7 mL of freshly prepared native and modified *D. hispida* starch Pickering emulsions were placed in a glass vial and were let to stand for 5 weeks. The emulsion layer of each Pickering emulsion was observed for each week and calculated according to the following equation by Li et al. (2020b):

$$\text{Emulsion Index (\%)} = \frac{\text{height of emulsion layer}}{\text{height of total volume}} \times 100 \quad (3)$$

Centrifugal test

Approximately 7 mL of native and modified *D. hispida* starch Pickering emulsions were placed in centrifuge tubes before being centrifuged at 500, 1000, 1500, 2000, and 2500 rpm for 10 minutes. The Pickering emulsion samples were then left to stand for 10 minutes, before calculating the creaming index as follows in equation 3.

Rheological properties

By following a method by Yu et al. (2019), the rheological behaviour of the Pickering emulsions was evaluated using a rheometer (DHR-3, TA instruments, USA) at 25 ℃ with a 40 mm pallet plate geometry. After placed on the plate, the emulsions were left for six minutes, for equilibrium of temperature. The Pickering emulsions' steady flow tests were conducted at shear rates of 0.1 to 300 s^{-1}. The linear viscoelastic region was used for all dynamic measurements. The strain was set at 0.1% and the frequency ranged from 0.1 to 100 rad/s. The storage modulus (G′) and loss modulus (G″) were recorded versus frequency.

Colour analysis

Both the powder and emulsions of both native and modified *D. hispida*'s starches were analysed using Chromameter (Konica Minolta DP-400, Japan), where the L*, a*, and b* readings were taken and recorded.

Statistical analysis

The investigations were conducted in triplicates and mean values were recorded. The tests of significant differences between the mean values were analysed using One-way Anova (Tukey Pairwise Comparison) at a significance level of 95% using Minitab software.

Results and Discussion

Degree of substitution of modified *D. hispida* starches

The degree of substitution (DS) denotes the average number of hydroxyl groups substituted per anhydrous glucose unit (AGU) in starch. For the starch modified

using OSA, the DS can be defined as the average number of hydroxyl groups that are substituted by the OSA group per glucose unit in starch (Lin et al., 2018). Determining the DS of starch is put on great importance not only to the emulsification ability of the starch and the stability of emulsion, but also for determining the digestibility of the starch (Lin et al., 2018). The increase of the DS for starch modified with OSA was found to possess enhanced emulsifying capacity of the starch and increase the stability of its emulsion (Lin et al., 2018).

The DS of native, OSA-modified and dual-modified *D. hispida* starches due to the response with OSA addition are shown in Table 1. An increase in the DS of both modifications (OSA and dual modified) was identified, with the DS from the OSA modification showing a higher DS value. However, the degree of substitution between both modifications and the native starch is not significantly different (p= [0.096], 95% confidence interval). This showed that the DS of the modified starches were differ due to different pre-treatment of starch, in which the dual-modified starch has undergone heat treatment before being esterified with OSA. The heating of the native *D. hispida* starch allows the starch to be gelatinised, thereby leading to the destruction of the molecular bonds between amylose-amylose, amylopectin-amylopectin, and amylose-amylopectin, which solubilises the starch molecules. This treatment changes the size of the granules, changes their molecular weight, and alters the physical and chemical characteristics of the modified starch.

Table-1. Degree of substitution for native, OSA-modified and dual-modified *Dioscorea hispida* starches.

Starch sample	Degree of substitution
Native	-
OSA	0.0013 ± 0.0299^{a}
Dual	0.0686 ± 0.0023^{a}

Values presented in the same column with the same superscripts are not significantly different ($p < 0.05$). (-) indicates no degree of substitution.

Heat treatment is commonly used to increase the entry of OSA into the starch granules, while also increasing the reaction between OSA and the hydroxyl group of starch (Dewi et al., 2022) within the Dual-modified starch. This study is in agreement with the study from Li et al. (2020a), Dewi et al. (2022), which found higher DS in OSA esterified starch compared to the native starch. This indicates an increase of hydroxyl groups substitution with OSA per glucose unit in the esterified starches compared to native starches. The DS of *D. hispida* starch with OSA modification, however, are higher when no heat-treatment is applied before the esterification with OSA. This can be due to alteration of the starch's crystalline structure, which hinders the penetration and substitution of OSA, as heat treatment can cause damage and cracks to the starch granules' surface (Subroto et al., 2024). Nonetheless, the DS depicts the entire extent of starch modification as the values can explain the existence of OSA groups within the starch modification proximity, where the hydroxyl group of the starch has been partially substituted with hydrophobic substituent, hence allowing for the appearance of amphiphilic character and interfacial properties (Dewi et al., 2022).

FTIR measurement

The FTIR spectroscopy is used to identify any changes in its chemical structure and functional groups resulting from modifications of the starches. New bonds or shifts in the characteristic absorption peaks like amine groups or ester carbonyl groups indicate successful chemical modification. In the region of 400 to 4000 cm^{-1}, a variation in the different stretch absorbance for all starches' modifications was shown against the native starch. Generally, peaks between 3000 to 3500 cm^{-1} indicates the existence of hydroxyl group, peaks between 3440 to 2930 cm^{-1} indicating stretching vibrations by O-H and C-H groups, peak at 1730 cm^{-1} relate to the stretching of ester carbonyl groups, between 2700 and 3700 cm^{-1} is the region of tension of polar hydrogen bonds within the starch (Quintero-Castaño et al., 2020). Dewi et al. (2022)

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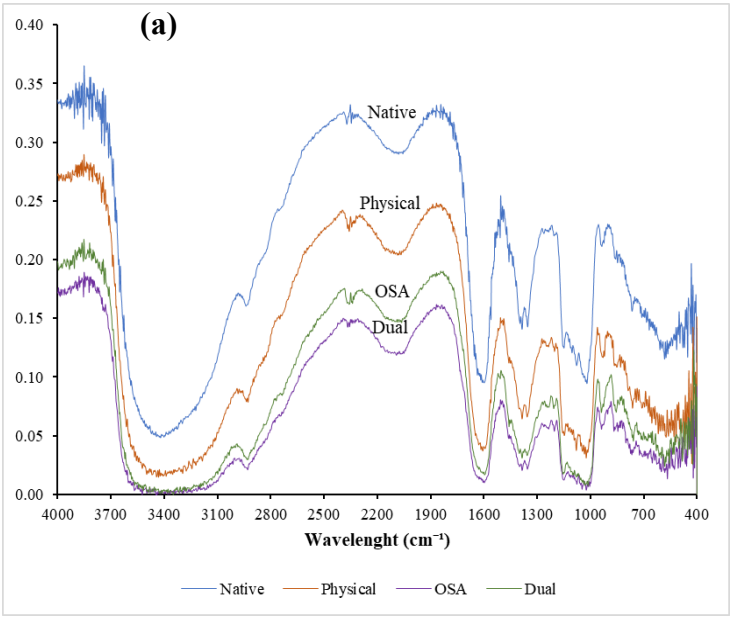
however, state that stretching vibration at wavenumber of 3400 cm^{-1} shows presence of O-H, $-CH_2$ at the peak of 2932 cm^{-1}, 800 to 1200 cm^{-1} for crystalline structure and short-range order of starch, and 995 cm^{-1} for short range of starch. Meanwhile, at 1407 till 1022 cm^{-1} absorption, bands are related with crystalline and amorphous structure of starch respectively, 995 cm^{-1} for intramolecular hydrogen bonds (hydroxyl group at C-6), and short-range molecular order alteration of double helices at ratio of 1022/ 995 cm^{-1} (Zhang et al., 2021a, Dewi et al., 2022).

Figure 1(a) illustrates the Fourier Transform Infrared spectroscopy (FTIR) for *D. hispida* starch with different modification techniques. FTIR was used to verify the existence of structural alteration of *D. hispida* starches. The FTIR spectra of physically modified, OSA modified and dual modified *D. hispida* starches were closely identical to the native starch, hence indicating that the modification has no influence on the skeleton of the starch. The bands appeared approximately at 2732 cm^{-1} for all starches are the region of tension of the polar bonds. Meanwhile, at peaks of 2353 cm^{-1} and 2091 cm^{-1}, progressive decrease in intensity can be observed with highest to lowest, native starch, physically modified starch, chemically modified starch and lastly dual modified starch, believed due to the change of the starch structure after modifications. This band reflects stretching of C-H and O-H correspondingly, which is supported by the finding from Hazrati et al. (2021) that stressed the peaks found between 1630 to 2930 cm^{-1} in *D. hispida* starches to reflect on the C-H and O-H band stretching. The chemical modifications of modified starches can be reflected in bands located at 1566 and 1557 cm^{-1}, where these weak bands only appear in spectra of modified starches (Quintero-Castaño et al., 2020).

Overall, this FTIR analysis discovered that the overall polysaccharides backbone of *D. hispida* starches remains intact after modifications. Subtle changes in the band density and the appearance of the new absorption bands confirm that the chemical functionalisation and structural rearrangement of the starch molecular interactions has been achieved successfully.



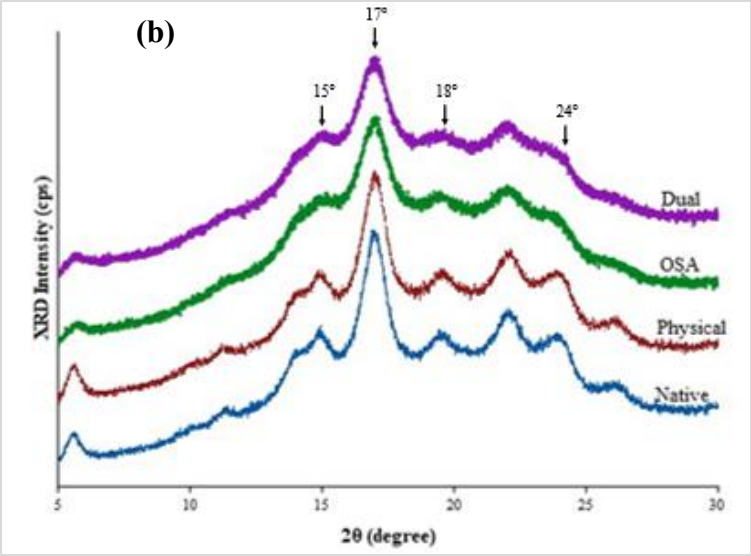


Figure-1. (a) Fourier transform infrared (FTIR) spectrum and (b) X-Ray diffraction diagram of native, physical, OSA and dual modified *D. hispida* starch granules.

X-ray diffraction (XRD)

The XRD test has been conducted to assess any changes in the crystalline structure and the degree of crystallinity that has been affected by the modification processes. XRD diagram and relative crystallinity of all *D. hispida* starch samples are shown in Figure 1(b). Both native and modified starches of *D. hispida* presented an A-type crystalline pattern, with characteristic peaks appearing at 2θ of 15˚, 17˚, 18˚ and 24˚. This is similar to findings by Li et al. (2020a) which found that A-type crystalline is found typically when peaks appear at 2θ of 15˚, 17˚, 18˚ and 23˚ for native corn and porous starch, both with modifications with OSA, suggesting that the starches cannot be destroyed by modification of OSA. Meanwhile, a study by Hazrati et al. (2021) found a similar peak, revealing that the starch patterns are consistent. In this study, all of the starches show high-intensity peaks at 2θ near 17˚ which indicates the presence of well-ordered crystalline material, with both native and physically modified *D. hispida* starches appearing to be the highest among all. This might be distributed to the contents of components like lignin, cellulose, and hemicellulose (Hazrati et al., 2021). Hazrati et al. (2021) and Han et al. (2012) both suggest that the treatments on starch are capable of destroying the crystalline region of materials due to the high energy utilized between the granules and water molecules during the reaction. This study found that the crystalline region is still preserved, with only little alteration after modifications. The crystallinity of the starch sample for both OSA and dual modifications, however, can be observed to decrease, likely due to the substitution of hydroxyl groups in the starch structure with OSA groups during the modification process. This is consistent with the finding by Zhang et al. (2021a) and Wang et al. (2022) that found the decrease in crystallinity with increasing DS by OSA modification, hence indicating that the esterification reaction occurs in the amorphous region instead of the crystalline region.

The XRD analysis demonstrated that the samples retained A-type crystalline structure after modifications. This indicates that the fundamental crystalline polymorph of the starch was preserved. Despite this, the relative crystallinity after the OSA and dual modifications shows partial disruption of the ordered crystalline domain due to the esterification and molecular substitution.

Morphology (SEM)

The surface morphology and granular structure of the starches were observed for any physical changes caused by the modifications, as any changes correlate with the functional properties of the starch like gelatinisation behaviour, viscosity and digestibility. The analysis also assists in confirming successful modifications at microscopic levels. The shape of the native *D. hispida* starch granules is mainly in a polygonal shape, as shown in Figure 2(a-d), similar to

the finding from Abdul Hamid et al. (2020). In this study, there were changes on the surfaces of the *D. hispida* starches, except that the native starch surface is much rougher than the modified starches. For both physical and dual-modified starches, the edges of the granules are smoother. This might be due to the preheat treatment applied to modify these starch granules. Studies have found that heat treatment disrupts the starch crystallites, promotes amylose-lipid interactions, and also amylose-amylose and/or amylopectin-amylopectin chains interactions, causing variations in structure, granule morphology, crystallinity, and functionality of starch (Nguyen et al., 2019).

Furthermore, this study also found that the size range for the starch is significantly different (*P*= [0.012], 95% confidence interval). Analysis using the Tukey method (95% CI) found that the native starch of *D. hispida* is not significantly different from all its modified starches, except for OSA-modified starch (Table 2). This is in agreement with the study by Zhang et al. (2021b) which found that the addition of OSA enhanced the number and thickness of the starch granules, hence proving that the addition of OSA in *D. hispida* native starch helps in the size increase of the starch granules Similarly, finding by Falade and Ayetigbo (2021) found that, modifications by annealing, acid-hydrolyzation and citric-acid substitution resulted in a shift in the distribution of starch granules by introducing some size reduction effects on the starch granules of white, water and yellow yam starches. Furthermore, it is expected that physical modification would increase the starch granules' size significantly due to the swelling. However, this study found that there is no significant difference between the native and physically modified *D. hispida* starch. These findings are inconsistent with the findings by Deng et al. (2021) and Torrejon et al. (2023), in which heat treatment led to the expansion of starch granules of potato starch and corn starch due to rupture, adhesion and partial gelatinisation. This suggests that different treatments alter the starch granules differently according to their variety.

Overall, the SEM analysis revealed that the polygonal morphology of the starches is retained after modifications, with the surface smoothness and granule sizes altered depending on the modification methods. It also can be concluded that the OSA modification exerted a stronger influence on the granule sizes compared to the physical treatment. These findings suggested that the morphological responses of the starch granules are highly dependent on the modification methods.

Table-2. Particle size (μm) of *Dioscorea hispida* starch granules at different modification methods.

Starch	Size (μm)
Native	2.71 ± 0.6^{a}
Physical	3.16 ± 0.65^{ab}
OSA	3.52 ± 0.42^{b}
Dual	3.04 ± 0.57^{ab}

Values presented in the same column with different superscripts were significantly different ($p<0.05$).

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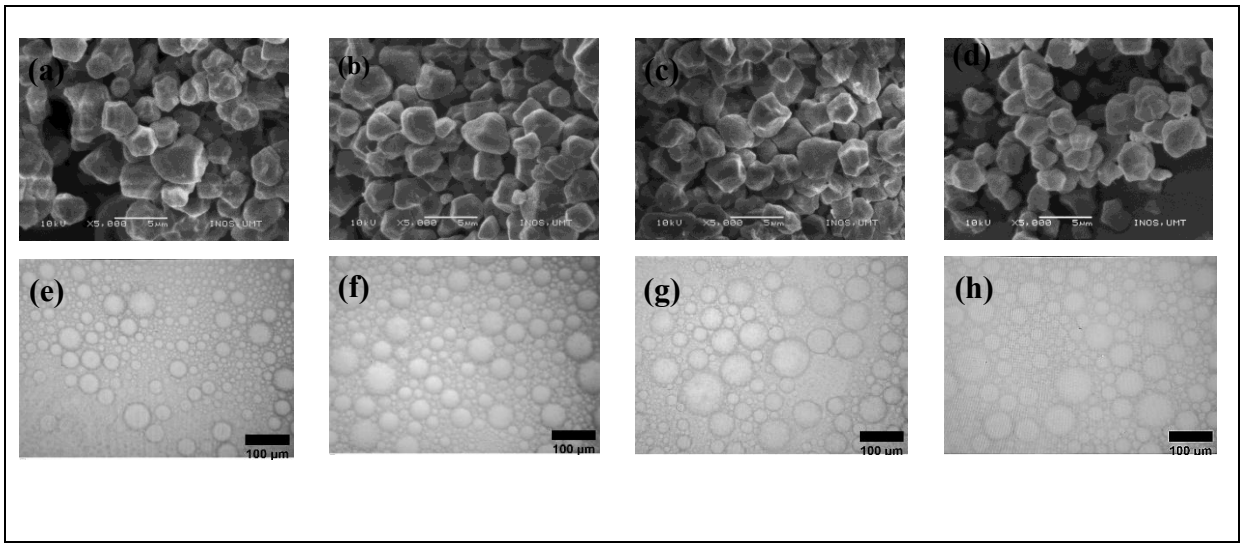

Figure-2. Scanning electron micrographs (a-d) and microstructures of Pickering emulsions (e-h) of native and modified starches of *D. hispida* under x5000 magnification; (a,e) native; (b,f) physical; (c,g) OSA; (d,h) dual-modified starches under 10x magnification.

Thermal properties

Changes in the thermal properties of the starch correlate with the functional attributes of the starch like gelatinisation behaviour, thermal stability and overall performance during cooking and processing, revealing differences between each modification. The thermal properties of *D. hispida* starches (Table 3) were analysed using DSC, focusing on onset gelatinisation temperature (T_o), peak gelatinisation temperature (T_p), final gelatinisation temperature (T_c), and gelatinisation enthalpy (ΔH). Native and physically modified *D. hispida* starches exhibit the highest onset temperature, meaning that they retain a more stable and ordered crystalline structure. This is supported by the XRD result (Section 3.3), which shows that the native *D. hispida* has the highest crystallinity peak. Both OSA and dual modified *D. hispida* starches, however, show low T_o compared to other samples, 72.63 ℃ and 72.08 ℃ respectively, due to the introduction of hydrophobic OSA groups in the starches, which disrupt the crystalline structure. This is supported by the XRD spectra, which displayed that OSA and dual-modified *D. hispida* starches had the lowest crystallinity, indicating that these starches are more easily gelatinised. Meanwhile, there is no significant difference for the ΔH for all starch modifications compared to native *D. hispida.* The ΔH was found to be relatively lower (1.98 J/g) compared to the study Abdul Hamid et al. (2020) for native *D. hispida* starches at 4.11 J/g to 4.16 J/g, hence suggesting that less energy is involved or absorbed during the phase transition. Furthermore, the *D. hispida* starch treated with dual modification indicated the lowest ΔH compared to other samples, probably due to the depolymerization process of the starch (Basilio-Cortés et al., 2019). This finding is aligned to the finding on corn starch by Basilio-Cortés et al. (2019) in which both hydrolysed with or without succinylation show lower gelatinisation enthalpy compared to the native corn starch. These results suggest that different starch modifications can alter the starch granule structure but also alter the thermal properties of the starches.

Table-3. Onset of gelatinisation temperature (T_O), peak gelatinisation temperature (T_P), final gelatinisation temperature (T_C), and gelatinisation enthalpy (ΔH) of native and modified *D. hispida* starches (physical, OSA and dual modification).

Starch	Onset Gelatinisation Temperature, T_O (℃)	Peak Gelatinisation Temperature, T_P (℃)	Final Gelatinisation Temperature, T_C (℃)	Gelatinisation Enthalpy, ΔH (J/g)
Native	75.50 ± 0.01[a]	78.13 ± 0.08[a]	82.56 ± 0.83[a]	1.98 ± 0.44[a]
Physical	75.14 ± 0.59[a]	78.24 ± 0.18[a]	82.79 ± 0.38[a]	1.46 ± 0.57[a]
OSA	72.63 ± 0.77[b]	74.41 ± 1.63[ab]	79.84 ± 1.61[a]	1.06 ± 0.15[a]
Dual	72.08 ± 0.19[b]	74.89 ± 0.15[b]	80.11 ± 0.32[a]	1.23 ± 0.53[a]

Values presented in the same column with different superscripts were significantly different ($p<0.05$).

Microstructure of Pickering emulsion

The interaction of starch particles at the oil-water interface can be revealed through microstructural analysis to discover the size, shape and distribution of the adsorption of the starch granules on the droplet surfaces. Observation of microstructure helps clarify the formation of protective layers of starch particles to prevent droplet coalescence and improvement of the stability of Pickering emulsions. In Figure 2(e-h) and Table 4, the droplet size of the Pickering emulsion stabilised by native starch was relatively the smallest (13.81 μm) with the presence of free oil in the emulsion system. The droplet size of the physically modified Pickering emulsion was observed to be larger (23.91 μm) than the native Pickering emulsion, with more free oil distribution within the emulsion. Both OSA-modified Pickering emulsion and dual-modified Pickering emulsion had the largest and large droplet sizes with 36.34 μm and 23.84 μm, respectively. Although most of the starch granules bound jointly at the droplet interface, some of the oil was not tightly adsorbed by the starch granules, resulting in coalescences. Greater interface area by smaller droplet is correlated with a stronger emulsifying power of the emulsifier. Similarly, the variation of droplet size able to represent the size of the interface region that can be stabilised by an emulsifier (Wang et al., 2023). Therefore, according to the droplet size, most of the starches have a strong emulsifying ability. Despite this, their emulsifying efficiency and stabilisation mechanisms differ depending on the modification methods.

Table-4. Droplet size of Pickering emulsion stabilised by *D. hispida* starch at different modification methods.

Starch	Diameter (μm)
Native	13.81 ± 4.91[a]
Physical	23.91 ± 5.52[b]
OSA	36.34 ± 20.32[c]
Dual	23.84 ± 9.73[b]

Values presented in the same column with different superscripts were significantly different ($p<0.05$).

Creaming index (CI)

The creaming index (CI) of the Pickering emulsions stabilised by modified starch is crucial in determining the physical stability of the emulsion over time, specifically on the resistance towards phase separation of the oil droplets and emulsion. Additionally, the effectiveness of the stabilisation of Pickering emulsion might vary depending on the formation of the dense interfacial film that helps in preventing the droplet aggregation and coalescence. The CI trends of Pickering emulsions stabilised by native and modified *D. hispida* starches over a four-week storage period are presented in Figures 3(a) and Figure 3(c-f). The native starch exhibited no significant changes in CI

with a consistent value from week 0 (95.65%) to week 4 (95.24%), indicating high stability throughout the storage period. Similarly, the physically modified starch demonstrated no substantial variation, with CI values ranging from 95.52% at week 0 to 91.80% at week 4. In contrast, the OSA-modified starch showed a declining trend in CI, with no significant difference observed between week 0 and week 3, followed by a gradual decrease thereafter. The dual-modified starch also exhibited a decline in CI over time.

Overall, these findings suggest that native and physically modified *D. hispida* starches contribute to greater emulsion stability, while OSA and dual modifications result in reduced stability during storage. Notably, the CI of all emulsions generally decreased over time, except for the emulsion stabilised by native starch, which remained stable. It was expected that the smallest droplet size would lead to better emulsion stability, as mentioned by Kong et al. (2025). This observation is supported by the findings in Figure 3(c-f) and Table 4, which indicate that emulsions with both the large (dual-modified) and largest (OSA-modified) droplet sizes exhibited the weakest stability, whereas the native starch, with the smallest droplet distribution, maintained superior stability from week 2 to week 4. In addition, the Pickering emulsion stabilised by dual-modified starch exhibited large droplet size (23.84 µm), demonstrating the lowest CI, suggesting instability likely due to droplet coalescence. These findings indicate that droplet size alone is not a parameter of emulsion stability for *D. hispida* starch-based Pickering systems, as the interfacial properties and particle adsorption strength also significantly influencing the long-term stability of the Pickering emulsion systems.

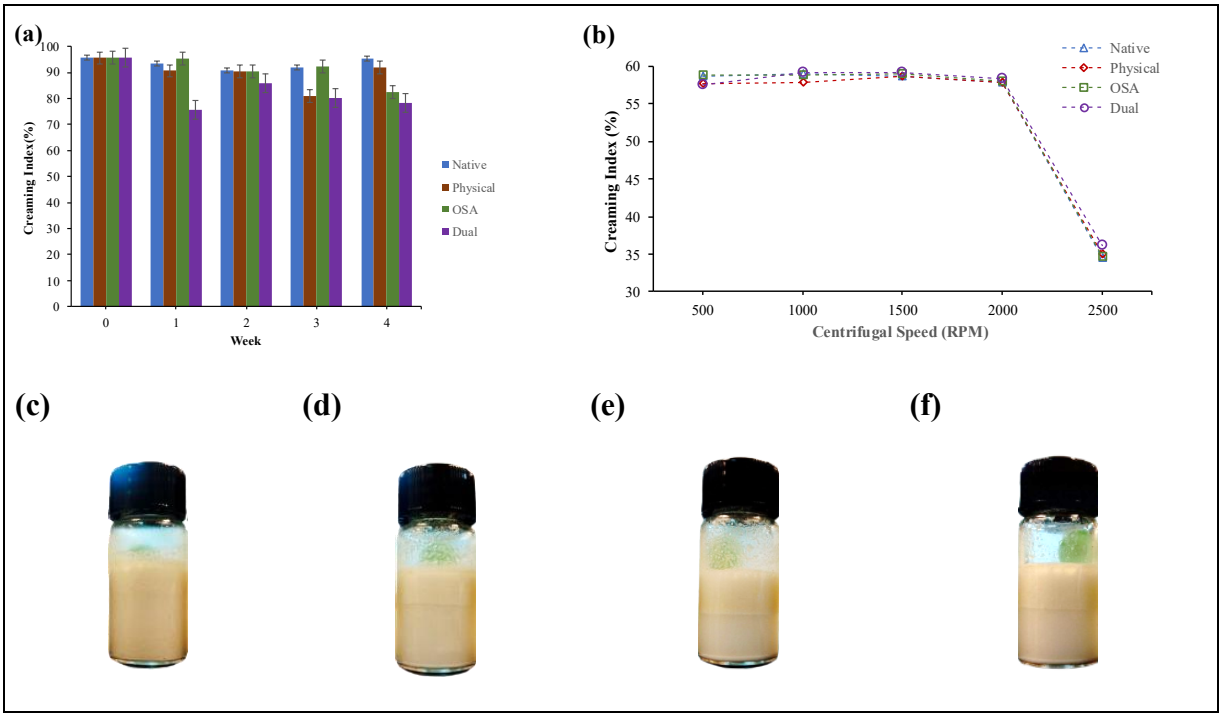

Figure-3. Figures of creaming index of Pickering emulsions stabilised by native and modified *D. hispida* starches (a); Creaming index (%) of *D. hispida* starch stabilised Pickering emulsions determined by centrifugation at varying speeds (500, 1000, 1500, 2000, and 2500 rpm) (b); and Pickering emulsions stabilised by *D. hispida* native starch (c), physical (d), OSA (e), and dual modified starch (f) in week 4 of emulsion index observation.

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Centrifugal test

The centrifugation test was conducted to investigate further the physical stability of the Pickering emulsion under gravitational force. The application of the strong force helps speed up the creaming processes, allowing for rapid evaluation of emulsion stability beyond normal storage period. The CI (%) of each Pickering emulsion after centrifugation is shown in Figure 3(b). The results indicate that the CI remains relatively stable across lower centrifugal forces (500–2000 rpm), with an CI obtained of >55%, suggesting that starch granules as emulsifiers are effective in maintaining emulsion stability under mild gravitational forces. However, at 2500 rpm, a significant reduction in the CI was observed in all Pickering emulsions, indicating a disruption in the emulsion droplets by coalescence which results to phase separation due to the higher gravitational force.

During centrifugation, oil droplets, being of lower density than the continuous aqueous phase, migrates toward the axis of rotation. This behaviour may lead to coalescence, either as a result of droplet collisions or due to insufficient surface coverage by stabilising solid particles (Low et al., 2020). The spatial distribution of starch granules around the emulsion droplets is influenced by droplet size, whereby smaller droplets tend to be displaced further from the axis. A decline in the creaming index (CI) at an elevated centrifugal force of 2500 rpm is therefore anticipated, as higher speeds increase the likelihood of droplet coalescence and promote the tighter packing of particles at the droplet interface (Low et al., 2020). This shows that the Pickering emulsions become unstable at a centrifugal force of 2500 rpm, corresponds to the study by Touma et al. (2024), which found that the silicone oil is prone to coalescence under centrifugal force. The results imply that *D. hispida*-stabilized Pickering emulsions exhibit good resistance to mild centrifugal force, but reach their critical demulsification point at 2500 rpm, beyond which extensive coalescence and phase separation occurs.

Rheological properties

Viscosity

Evaluation on the flow behaviour and structural integrity of the Pickering emulsion is crucial to estimate the resistance towards droplet coalescence and phase separation. Additionally, the change in viscosity reflects the degree of starch modifications and its impact on the particle wettability and network formation in the continuous phase. As shown in Figure 4(a), the apparent viscosity of all emulsions decreased with increasing shear rate, hence indicating a typical shear-thinning behaviour of the Pickering emulsions. There is also a similarity in the other emulsion system (Wang et al., 2023; Dong et al., 2023; Hu et al., 2022). These findings suggest that both native and its modifications significantly increased the shear-thinning behaviour of the Pickering emulsions. For instance, a study by Wang et al. (2023) demonstrates that the apparent viscosity of Pickering emulsions stabilised by rice, waxy rice, wheat, and pea was decreased with increasing shear rate, showcasing typical shear-thinning behaviour of pseudoplastic fluid. Table 8 also highlights that Pickering emulsions stabilised by dual modified starch show the lowest shear thinning behaviour. Although the physically modified starch exhibited the highest viscosity due to its thickening properties by gelatinisation process, the dual modified starch exhibited the opposite behaviour. This resulted to its lowest CI, as shown in Figure 3(b), indicating the highest degree of phase separation. The free oil obtained from this phase separation contributed to the reduction in viscosity. Study by Zhai et al. (2023) found that small droplet size emulsions have significant influence on the shear viscosity due to the strong hydration effect of the small droplets, leading to a more compact network structure (Lv et al., 2023; Zeng et al., 2023).

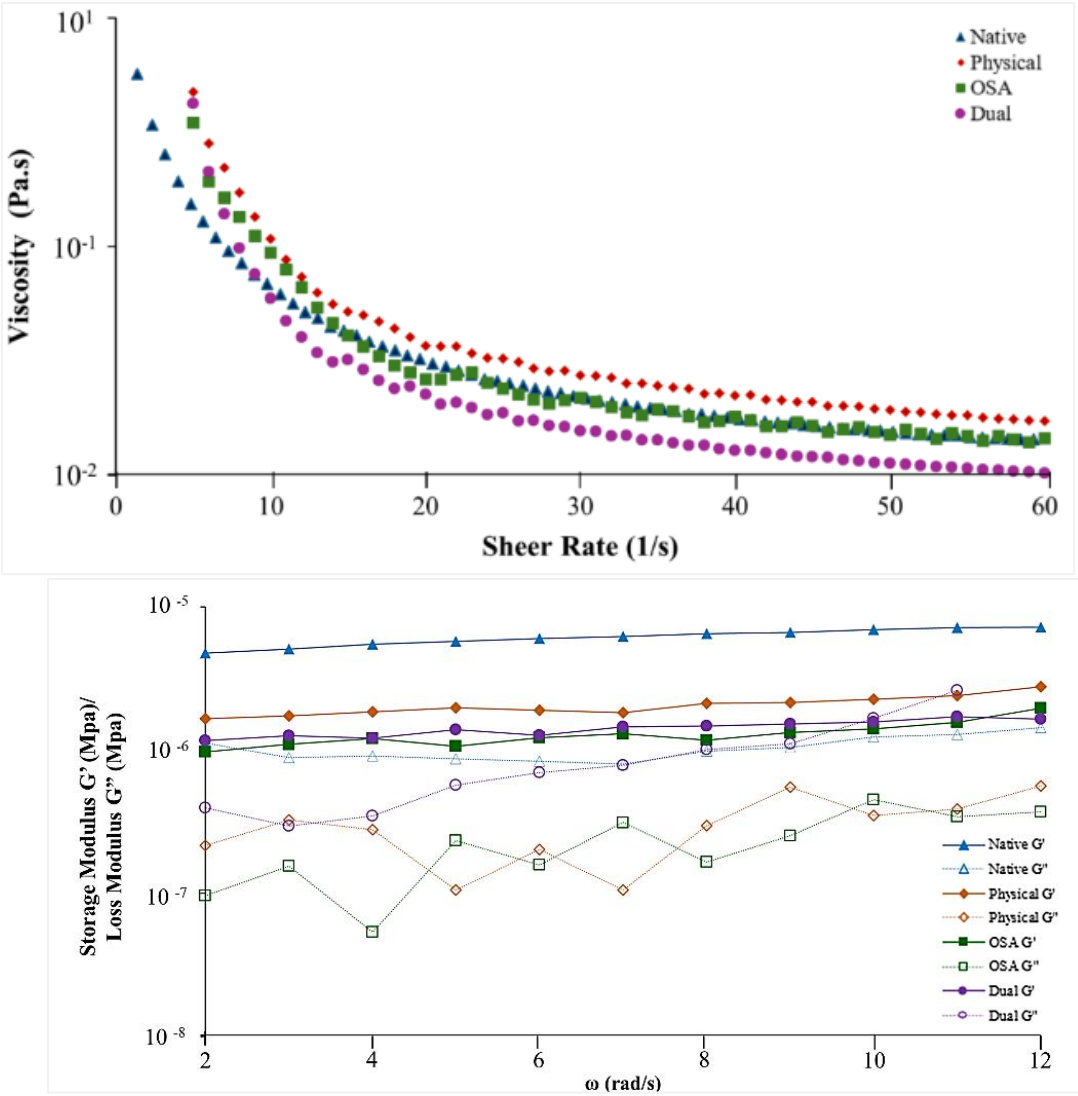

Figure-4. Viscosity profile of the native, physical, OSA and dual modified *D. hispida* starch Pickering emulsions (a); and Storage modulus (G') and loss modulus (G") of the native, physical, OSA, and dual-modified *D. hispida* starch stabilised Pickering emulsions (b).

Viscoelastic behaviour

Evaluation on the viscoelastic behaviour of Pickering emulsion is crucial in assessing the structural strength and stability of the Pickering emulsions over time. G' is denoted as the solid or elastic form of the material's stored energy, meanwhile, G" indicates the liquid or viscous form of the material's stored energy. Guleria and Yadav (2022) indicate G' as a degree of elastic response of a material and measured stored energy in a material per deformation cycle, and G" as a measure of viscous response of the material and measured the energy dissipated from the material per deformation cycle. The storage modulus (G') and loss modulus (G") against angular frequency (ω) observed within the Pickering emulsions stabilised by *D. hispida* are shown in Figure 4(b). This study found that all Pickering emulsions stabilised by native and modified *D. hispida*'s starch exhibited higher G' than the G" value. This suggests that all Pickering emulsions of *D. hispida* exhibit elastic properties (G'>G"). This finding is equivalent to the finding by Benyaya et al. (2024), where polymeric particle emulsions were relatively self-sufficient of angular frequency over a wide range. These attributes are, however, common for Pickering emulsions with elevated oil volume fractions, which is also part of the characteristic of

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highly flocculated elastic structure (Benyaya et al., 2024; Wei and Huang, 2019; Jia et al., 2019). Pickering emulsion stabilised by native *D. hispida* showed the highest G' value compared to other samples, indicating it is less dependent towards the frequency sweep. This is mainly contributed by deformation of droplet interfaces, as the droplet tension keeps the interfacial energy caused by the combining of solid particles adsorbed at the oil-water interfaces (Taghavi et al., 2024). These results affirmed a higher emulsifying stability of native starch. The G' significantly decreases, suggesting a deformation of stronger gels after modification and vice versa. The decreasing value in G' also might be credited to the degree of granular swelling, which failed to fill the available volume of the system completely (Singh et al., 2003; Eliasson, 1986).

Therefore, these findings demonstrated that the dynamic rheological properties of the Pickering emulsions are influenced highly by the starch modifications and the interfacial microstructure compared to dependence on the frequency only.

Colour analysis

The differences in colour for the starch powders and emulsified starches of both native and modified *D. hispida* were investigated in this study. Evaluation of the color of starch powder and its Pickering emulsion is important to evaluate the visual and aesthetic qualities of the products. The colour parameters of the starch powders and emulsions were summarized in Table 5. The table shows the colour values L* (lightness), a* (redness), and b* (yellowish) difference between the samples, both for powders and emulsion. It is found that between the samples in the form of powders, OSA-modified starch showed the highest lightness value, L* (80.55) compared to the lowest L* value in native starch (76.04). a* value for OSA-modified starch is also found to be the lowest (+0.02) compared to other starch powders. Meanwhile, for the b* value, the dual modified starch value is the lowest (+2.39), with the highest value found in physical starch powder (+4.99). Hence, the lightest powder starch is found to be OSA-modified starch, with the lowest yellowish colour and has moderate redness colour. The native starch powder was found to have the lowest lightness, with the highest yellowish and in between redness compared to other starch powders.

For the Pickering emulsions, the dual-modified emulsion has the lowest L* value (77.00). Meanwhile, the lowest a* value was found in the OSA-modified emulsion with the value of -1.18, and the lowest b* value was found in the dual-modified emulsion (+4.01). The highest L* and a* values were both found in native starch emulsions (79.85 and -0.66, respectively), however, the highest b* value was found in the physically modified emulsion with a value of +5.55. This shows that the native emulsion has the lightest colour among all emulsions; meanwhile, the least yellowish emulsion was found to be the OSA-modified starch emulsion. Among all the colour analyses between both different attributes (L*, a* and b*) values in both powder and emulsified starch, all means are significantly different, except for the b* value in the emulsified starch, where both native and physically modified starch emulsions were tied with the same letter showing that it does not have any significant difference. However, all the samples for the colour analysis were indicated to have significant differences, as all the P-values for each column in both starch powders and emulsions are equal to 0, where $p<0.05$.

Table-5. Colour parameters of *D. hispida* starch powder and *D. hispida* starch stabilised Pickering emulsions at different modification methods.

Type of Starch	Powder			Emulsion		
	L*	a*	b*	L*	a*	b*
Native	76.04±0.02^{a}	+0.52±0.01^{a}	+4.39±0.01^{a}	79.85±0.06^{a}	-0.66±0.01^{a}	+5.51±0.02^{a}
Physical	77.77±0.01^{b}	+0.36±0.01^{b}	+4.99±0.01^{b}	79.39±0.05^{b}	-0.91±0.02^{b}	+5.55±0.01^{a}
OSA	80.55±0.01^{c}	+0.02±0.02^{c}	+2.94±0.01^{c}	78.56±0.06^{c}	-1.18±0.01^{c}	+4.66±0.02^{b}
Dual	79.67±0.01^{d}	+0.19±0.03^{d}	+2.39±0.02^{d}	77.00±0.02^{d}	-0.97±0.01^{d}	+4.01±0.03^{c}

Values present in the same column with different superscripts were significantly different ($p<0.05$).

The findings in this study on the colour analysis of emulsions for both native and modified starches of *D. hispida* are comparable to the findings by Abdul Hamid et al. (2020), which found that the L* value for the yellow *D. hispida* is 76.10, which is almost similar to the L* value of the native powder starch of *D. hispida* (76.04). However, the a* and b* values for this study are lower than the findings from Abdul Hamid et al. (2020) which is +1.32 and +5.88. This is expected since the findings from Abdul Hamid et al. (2020) were conducted using raw *D. hispida;* meanwhile, this study used samples from different modifications. It is presumed that the parameters of a* and b* for each sample differ due to the isolation and modification methods of starch.

Conclusion

In this study, it was found that the starches displayed well-ordered crystalline material with high-intensity peaks at 2θ near 17˚. However, a notable reduction in crystallinity was observed in starches subjected to octenyl succinic anhydride (OSA) and dual starch modifications. Native and modified starch granules displayed a polygonal shape, with sizes ranging from 2.71 to 3.52 µm. Among the samples, native starch demonstrated the highest gelatinisation temperature. In the Pickering emulsion, dual-modified starch exhibited large droplet size (23.84 µm), with the lowest CI. Conversely, Pickering emulsions stabilised by native and physically modified *D. hispida* starches maintained good stability over the four-week storage period. Under low centrifugal force, all emulsion samples exhibited comparable stability (CI > 55%), along with shear-thinning and gel-like rheological behaviour. However, at 2500 rpm, all samples exhibited coalescence, indicating that this is the critical point at which *D. hispida* starch-stabilised Pickering emulsions begin to break down. These findings highlight the potential of *D. hispida* starches, particularly native and physically modified forms, as effective, natural stabilisers for Pickering emulsions, offering a cost-efficient and sustainable alternative for industrial applications.

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Contribution of Authors

Zaid NISM: Methodology, investigation, formal analysis, data curation, writing-original draft and validation.

Taghavi E: Conceptualization, writing- review and editing, supervision and validation.

Mehr HM, Seruji AZRA & Rasdi NW: Validation and writing-review and editing

Abdul Hadi N: Conceptualization, methodology, data curation, funding acquisition, supervision and writing-original draft.

All authors read and approved the final draft of the manuscript.

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