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Characterisation of the mucilage polysaccharides from *Dioscorea opposita* Thunb. with enzymatic hydrolysis

Running Title: Enzymatic hydrolysis of yam mucilage polysaccharides

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Abstract

The mucilage polysaccharides from *Dioscorea opposita* (DOMP) were extracted and treated with a single/dual enzymatic hydrolysis. The characterisation and viscosity were subsequently investigated in this study. DOMP obtained 62.52% mannose and 23.45% glucose. After single protease and trichloroacetic acid (TCA) treatments, the mannose content was significantly reduced to 3.96%, and glucose increased from 23.45% to 45.10%. Dual enzymatic hydrolysis also decreased the mannose and glucose contents to approximately 18%-35% and 7%-19%, respectively. The results suggest that enzymatic degradation could effectively remove the protein from DOMP accompanied by certain polysaccharides, especially mannose. The molecular weight, surface morphology, viscosity and particle sizes were measured. Enzymatic hydrolysis reduced molecular weight, decreased the viscosity, and increased the particle sizes, which indicates that the characterisations of DOMP samples were altered as structures changed. This study was a basic investigation into characterisation of DOMP to contribute to the processing of food by-products.

Keywords: Chinese yam, mucilage, polysaccharides, dual enzyme hydrolysis

Abbreviations:

CY, Chinese yam; DOM, *Dioscorea opposita* mucilage; DOMP, *Dioscorea opposita* mucilage polysaccharides; MW, molecular weight.

1. Introduction

Mucilage is defined as a gelatinous substance or a type of hydrocolloid with strong interactions between polysaccharides and proteins (Lai and Liang, 2012; Zeng et al., 2016). Mucilage polysaccharides are naturally occurring viscous colloidal dispersions with a high molecular weight (Singh et al., 2009; Han et al., 2016). Polysaccharides have been extensively used in the food industry for their functional properties, such as thickeners, gelling agents, stabilisers, interfacial agents, etc. (Stephen et al., 2006). According to Nayak et al. (2016), plant-extracted mucilage polysaccharides are non-toxic and safe materials to be used in the food industry as suspending agents, thickeners, emulsion stabilisers, water retention agents and film-forming agent, etc.

Dioscorea opposita Thunb., the Chinese yam (CY), is a tuber crop that has nutritional and economic significance in China (Zhang, et al., 2014). According to previous studies, *Dioscorea opposita*, which is an important edible and pharmaceutical food in China, contains various chemical components and nutrients, including polysaccharides, amino acids, flavonoids, allantoin, dopamine, and batatasin (Chen et al., 2015; Yang et al., 2008; Wang et al., 2006). *Dioscorea opposita* has bioactivity and health benefits, such as enhancing immunity, lowering blood sugar, and has pharmacological functions, including treating haemorrhoids, sore throat and struma, lung diseases and the pancreas disease, etc. (Chan & Ng, 2013; Ma et al., 2017).

The dried slices of CY are frequently used as traditional Chinese medicine

because fresh *Dioscorea opposita* has seasonal harvesting and short storage life. During the industrial process of dried slices from the fresh tuberous rhizomes of *Dioscorea opposita*, the mucilage (DOM) has always been ignored and discarded in line production, which has resulted in a large waste of resources (Li et al., 2014; Hou et al., 2002). Therefore, extracted *Dioscorea opposita* mucilage polysaccharides (DOMP) has a great potential for using in food applications and functional food.

Currently, enzymatic hydrolysis has been used to improve or customise the properties as well as modify the structures of existing polysaccharides (Cheng & Gu, 2012; Zeng & Lai, 2016). Kim et al. (2013, 2014) reported that structural modification by enzymes changed the physical behaviour of their model pectin. Enzymatic hydrolysis also lowers the molecular weight or debranches the lateral chains of polysaccharides, which could lead to valuable polysaccharide applications (Leathers et al., 2015). Jo et al. (2016) investigated the nutritional quality and the development of new dietary applications of sweet potato as well as value-added products generated though enzymatic modification of starch. Despite the relatively low yields from enzymatic reactions, modified polysaccharides with a lower molecular weight still maintain their desired end-use properties (Cheng & Gu, 2012; Zeng & Lai, 2016).

The mucilage of *Dioscorea opposita* (DOM) was comprised of protein (≈ 2.78%), and polysaccharides, including glucose (≈ 49.50%), mannose (≈ 33.40%), galactose (≈ 10.90%), xylose (≈ 5.38%), arabinose (≈ 0.54%), and rhamnose (≈ 0.25%). The molecular weight (MW) of DOM was 143,700 Da (Ma et al., 2017). This study was

conducted to investigate the influence of enzymatic hydrolysis, including protease, α-amylase, mannanase, galactanase, xylanase, arabinase, and rhamnase, on the physicochemical features *Dioscorea opposita* mucilage polysaccharides (DOMP), such as viscosity. A viscosity study of DOMP could be used to explore the correlation between structures and functions. In this manner, enzymatically hydrolysed DOMP with specifics characteristic may meet the requirements for diverse by-products.

2. Materials and Methods

2.1. Materials

Fresh *Dioscorea opposita* Thunb. was purchased from Bao He Tang (Jiaozuo) Pharmaceutical Co. Ltd. in November, 2016. Protease (10 U/mg, purified from *Bacillus licheniformis*, Lot 90701), α-amylase (55 U/mg, purified from *Bacillus licheniformis*, Lot 111201b), endo-1,4-β-mannanase (417 U/mg, purified from *Cellvibrio japonicus*, Lot 90901b), endo-1,4-β-galactanase (506 U/mg, purified from *Aspergillus niger*, Lot 101001b), endo-1,4-β-D-xylanase (38 U/mg, purified from *Cellvibrio japonicus*, Lot 90601b), endo-arabinanase (15 U/mg, purified from *Aspergillus niger*, Lot 111201b), and endo-rhamnosidase (190 U/mg, purified from a prokaryote, Lot 110501b) were purchased from Megazyme International Ireland (Bray Business Park, Bray, Co. Wicklow, Ireland). All reagents and standard samples were purchased from Sigma-Aldrich Co. Ltd, USA, or Tianjin Kemiou Chemical Reagent Co. Ltd, China. All chemicals used were of analytical grade.

2.2. Extraction and enzymatic treatment of *Dioscorea opposita* mucilage polysaccharide (DOMP)

2.2.1. Extraction of DOMP

Dioscorea opposita mucilage (DOM) was extracted as previously described by Ma et al. (2017). Briefly, *Dioscorea opposita* were washed, peeled, and washed again in deionised water (pH 7.0, resistivity: 18 Ω·m). *Dioscorea opposita* was then sliced and ground in an industrial blender for 5 min. All portions were subsequently pooled and homogenised. After centrifugation at 4,000 rpm for 5 min, DOM was collected in the supernatant, and three volumes of ethanol were added for precipitation (24 h). *Dioscorea opposita* mucilage polysaccharide (DOMP) was then precipitated and collected by centrifugation (4,000 rpm for 5 min). The DOMP precipitant was lyophilised for 3 days to a constant weight and stored in vacuum desiccators over phosphorus pentoxide until they were used.

2.2.2. Preparation of DOMP samples with enzymatic hydrolysis

Enzymatic hydrolysis of DOMP was carried out according to the methods described by Zeng and Lai (2016) with modifications. DOMP was divided into two separated portions for various enzymatic hydrolysis procedures (flow chart shown in Fig. 1). The first portions of DOMP were used for protease hydrolysis. 4.00 mg DOMP were dissolved with 125 mL of 50 mM phosphate buffer (pH 7.0), followed by adding approximately 50 U of protease and incubating the solution at 37 °C for 2 h. 25.0 mL of 9.0% trichloroacetic acid (TCA) were then added to terminate the proteinase reaction. The mixture was subsequently centrifuged (6,000 rpm, 20 min), and the supernatant was dialysed against deionised water by using a dialysis membrane (MWCO, 500 Da, Solarbio Life Sciences, Beijing, China). Three volumes

of ethanol were subsequently added to the dialysed sample solution, and 24 h later, the precipitation (DOMP-NP) was collected and lyophilised to a constant weight after centrifuging (6,000 rpm, 20 min).

Another portion of DOMP was carried out for dual enzymatic hydrolysis procedures. The same protease hydrolysis procedure was performed as described previously, except that the proteinase treatment was terminated by heating at 70 °C for 20 min. After cooling, 108.9 U of α-amylase (Amase), 58.4 U of mannanase (Mase), 94.2 U of galactanase (Gase), 45.6 U of xylanase (Xase), 21.8 U of arabinanase (Arase), or 30.4 U of rhamnosidase (Rase) were added separately and incubated at 40 °C, 50 °C, 50 °C, 40 °C, 60 °C, and 50 °C, respectively, for 2 h. The reaction mixtures were then centrifuged, dialysed, precipitated with ethanol, and lyophilised as previously described to obtain the deproteinised DOMP with Amase (DOMP-Amase), Mase (DOPM-Mase), Gase (DOMP-Gase), Xase (DOMP-Xase), Arase (DOMP-Arase), or Rase (DOMP-Rase) hydrolysis, respectively. The samples were stored in vacuum desiccators over phosphorus pentoxide until they were used.

2.3 Characterisation of DOMP with enzymatic hydrolysis

2.3.1. Yield

4.00 mg of DOMP were used each time to modify the structure, and the final hydrolysed DOMP was lyophilised and weighed. Therefore, the yield (%) of enzymatically hydrolysed DOMP was calculated by the following formula:

$$\text{Yield (\%)} = \frac{\text{Weight of enzymatic hydrolysed DOMP samples}}{\text{Weight of DOMP (4.00 mg)}} \times 100\%$$

159 2.3.2. pH determination

160 Enzymatic hydrolysed DOMP samples (1% w/v) was prepared and a pH metre
161 (ZD-2A, Dapu Instrument, Shanghai, China) was used to measure the pH value of the
162 sample solutions. The mean value of three consecutive measurements was recorded.

163 2.3.3. Determination of monosaccharides

164 As previously described by Wang et al. (2016), 1-phenyl-3-methyl-5-pyrazolone
165 (PMP) derivatization and high-performance liquid chromatography (HPLC, Waters
166 1525, USA) was used for determination of monosaccharides with a Thermo
167 DOS-2-C18 column (4.6 × 250 mm, 5 μm). Nine standards (Ludger Co. Ltd)
168 including arabinose, rhamnose, galactose, glucose, mannose, xylose, ribose,
169 galacturonic acid and glucuronic acid were used to determine the monosaccharides in
170 hydrolysed DOMP samples. Chromatographic separation was carried out using 0.1
171 mol·L^{-1} phosphate buffer (pH 7.0) and acetonitrile at a ratio of 82:18 (v/v) as a mobile
172 phase at a flow rate of 1.0 mL·min^{-1}. The temperature of the column was maintained
173 at 25 °C and detected by variable-wavelength UV-visible detector (VWD) at 245 nm.

174 2.3.4. Determination of amino acids

175 As previously described by Waqas et al. (2015), an amino acid analyser (L-8900
176 Amino acid analyser, Japan) and Shim-pack amino-Na column (4.5 × 60 mm,
177 Shimadzu) were used to identify the amino acids in enzymatically hydrolysed DOMP
178 samples.

179 2.3.5. Determination of molecular weight (MW)

180 The weight-average MW (Mw) and MW polydispersity (Mw/Mn) were

measured using high-performance size-exclusion chromatography (HPSEC-MALLS-RID, Wyatt Technology Co., USA) with an OHpak SB-802.5 HQ column (8.0 mm × 300 mm, Shodex Co., Japan). The mobile phase was 0.1 M $NaNO_3$ at a flow rate of 0.5 $mL \cdot min^{-1}$, 50.0 μL of sample solutions (1.8 $mg \cdot mL^{-1}$) were injected, and the chromatogram was analysed using ARTRAV software (Wyatt Technology Co., USA).

2.3.6. Fourier transform infrared spectroscopy (FT-IR)

Enzymatically hydrolysed DOMP samples were analysed using FT-IR (Vertex 70, Bruker, Germany) with a spectral range of 4000 to 400 cm^{-1}. The transmission of the samples within 7 mm diameter KBr pellets was measured.

2.3.7. Scanning electron microscopy (SEM)

The hydrolysed DOMP samples were taken after freeze-drying and prepared by sticking them to one side of double-sided adhesive tape attached to a circular specimen stub, and sputter coated with vacuum spray gold. Moreover, freshly prepared solutions of hydrolysed DOMP samples were diluted, dropped on the prepared carbon-coated copper sheet and left to dry at room temperature (20 °C). The samples were completely dried and sputter coated with vacuum spray gold. A thermal field emission scanning electron microscope (JSM-7001F, JEOL Ltd., Japan) was used to inspect the morphology of enzymatically hydrolysed DOMP samples.

2.3.8. Particle sizes

The droplet diameters and zeta-potential of the solutions made by hydrolysed DOMP samples were investigated using Malvern zeta-potential (Malvern-NanoZS90,

Malvern Ltd., UK). To obtain comparable and representative data, the results were recorded as the averages plus or minus the standard deviation (repeated experiment number = 6, ± SD).

2.3.9. Viscosity

The viscosity of hydrolysed DOMP samples was measured by rotatory rheometer (TA-DHR2, TA Instruments, New Castle, Delaware, USA) with a 60 mm cone plate (2°). Flow sweep measurements were carried out to determine the viscosity with a shear rate in the range of 0.01 s^{-1} to 100 s^{-1}. Samples were loaded onto the rheometer, and it was allowed to equilibrate to the measuring temperature (25 ± 1 °C, ≈ 0.5 min). For each test, approximately 2 mL samples were transferred onto the plate.

3. Results and Discussion

3.1. Yield and chemical compositions of DOMP with enzymatic hydrolysis

The yield of *Dioscorea opposita* mucilage (DOM) and *Dioscorea opposita* mucilage polysaccharides (DOMP) were approximately 8.18% and 5.70%, respectively (Ma et al., 2017). Enzymatic hydrolysis treatment significantly reduced the yield of DOMP samples as expected (in the range of 3.40% to 4.46%, shown in Table 1). The yield of DOMP samples treated by protease alone was 3.61%, which was lower than other DOMP samples treated by protease + α-amylase (DOMP-Amase, 3.88%), protease + mannanase (DOMP-Mase, 4.15%), protease + galactanase (DOMP-Gase, 4.43%), protease + arabinase (DOMP-Arase, 4.46%), and protease + xylanase (DOMP-Xase, 3.92%). The results indicate that protein may interact with both the large and small polysaccharide fractions of DOMP and precipitate after

protease treatment. During dual enzymatic treatment, glycosidases, including mannanase, galactanase, xylanase, and arabinase, hydrolysed the precipitate after the proteinase reaction, and some of the monosaccharides, such as mannose and galactose, dissolved in the supernatant precipitated by the ethanol.

Protein content analysis in Table 1 revealed and compared both single enzymatic hydrolysis (DOMP-NP) and dual enzymatic treatments (DOMP-Amase, DOMP-Mase, DOMP-Gase, DOMP-Arase, DOMP-Xase, and DOMP-Rase). The protein content of DOMP-NP was approximately 4.62%, which was significantly higher than that of dual enzymatically hydrolysed DOMP samples. Particularly, DOMP-Amase contained the lowest amount of protein (approximately 0.06%), which suggested that protein could have interactions with 1-4-α-glucose. Moreover, approximately 0.99% of protein was obtained in DOMP with protease and xylanase treatment, which indicated that both protein and xylose may affect the linkage.

The main monosaccharides in DOMP were 62.52% mannose, 23.45% glucose, 9.30% xylose, and 3.33% arabinose. Single protease-treated DOMP terminated by TCA contained 45.10% glucose, 22.1% galacturonic acid, 19.64% galactose, 5.38% arabinose, and 3.96% mannose. Interestingly, the biggest difference is in the mannose content. The mannose contents in DOMP and DOMP-NP were 62.52% and 3.96%, respectively, which indicates that most mannose in the mucilage of *Dioscorea opposita* is more likely to be straight chains and serve as the structural skeleton of plant cells (Coultate, 2002). Schmitt et al. (2009) stated that protein and polysaccharides can be found in the same physiological environment and interact. The

dramatic reduction of mannose suggests that protein and mannose could interact together, and part of the polysaccharides was removed with proteins together, which was consistent with the results of Zeng et al. (2016). The galacturonic acid of DOMP-NP (22.11%) was significantly higher than DOMP (0.01%), which suggested that protease may break the structures of glycoprotein, and then trichloroacetic acid (TCA) could provide -OH or -OOH to increase the content of uronic acids. In addition, the content of arabinose, galactose, glucose and rhamnose in DOMP-NP increased dramatically compared to DOMP. The results show that during the deproteinisation, glucose, galactose, arabinose, and rhamnose were released due to the structural changes of polysaccharides. In other words, mannose, arabinose, galactose, rhamnose and glucose could exist in the linkages of proteins, and when glycoproteins go through deproteinisation, monosaccharides are released.

On the other hand, the dual enzymatically hydrolysed DOMP samples were used at 70 °C to inactivate protease treatment, and then were treated with monosaccharidase, which was terminated by TCA. Compared to DOMP, the contents of arabinose, galactose, rhamnose, and uronic acids in the dual enzymatically hydrolysed DOMP samples increased significantly. The contents of glucose in dual enzymatically hydrolysed DOMP samples decreased significantly. Compared to DOMP-NP, the arabinose, galactose, and mannose contents increased, meanwhile, the glucose and uronic acids content were extremely reduced. The results not only show that the polysaccharides and proteins were interacted together, but also reveal that samples with TCA termination of protease reaction leads to considerably different

monosaccharide contents in samples treated with high temperature inactivation.

3.2 Molecular weight (MW) and MW distributions of DOMP with enzymatic hydrolysis

The molecular weight, polydispersity (PDI, Mw/Mn), and distribution details are shown in Table 2. The molecular weight (MW) of DOMP-NP was 69,483 Daltons, higher than the rest of the dual enzymatically hydrolysed DOMP samples. The decrease in molecular weight implied that the protein might integrate with polysaccharides, and polysaccharides were partially removed from the structures (Zeng et al., 2016). Although dual enzymatic hydrolysis through the action of proteinase and monosaccharidase decreased the molecular weight of DOMP, the pattern of molecular weight distribution was intact.

The molecular weight was distributed into six sections, < 3, 3-10, 10-20, 20-100, 100-200, and > 200 kDa, and it was mainly in the range of 20-100 kDa. The molecular weight distributions of DOMP-Amase, DOMP-Gase, and DOMP-Rase were approximately 66.00%, 69.00%, and 66.00% respectively in the range of 20 to 100 kDa, which were higher than molecular weight in 20-100 kD of DOMP-NP (63.25%). Particularly, DOMP treated with both protease and mannanase had a higher yield (4.15%), lower molecular weight (63,923 Dalton), and a relatively low amount in the range of 20-100 kDa, which suggested that the proteinase cleaved the bound protein from polysaccharides, and smaller molecular weight of polysaccharides were precipitated. Interestingly, DOMP-Gase had a high yield (4.43%), lower molecular weight (65,122 Dalton), and 69.00% was in the range of 20-100 kDa. The MW

distribution of DOMP-Gase was 2.00% in 3-10 kDa, 11.50% in 10-20 kDa, 69.00% in 20-100 kDa, 16.25% in 100-200 kDa, and 1.25% were larger than 200 kDa, which demonstrated that the MW distribution was concentrated to 20-100 kDa. The results suggest that proteins in DOMP were hydrolysed, which led to two possibilities: first, some smaller molecular polysaccharides may co-precipitate from the addition of TCA due to the changes in pH and temperature, and second, proteinases may break the linkage of proteins and polysaccharides, and those proteins or polysaccharides were rearranged and aggregated (Zeng et al., 2016).

3.3. Characterisation of DOMP with enzymatic hydrolysis

3.3.1. FTIR

Fig. 2 shows the FTIR for enzymatically hydrolysed DOMP samples. The wide bands in 3700 - 3000 cm^{-1} indicate hydroxyl groups (-OH) (Andrade et al., 2015). DOMP with protease treatment presents the peak at 3306 cm^{-1}, which moved to 3420 cm^{-1} with dual enzymatic treatment and implied that dual enzymes with their optimal pH lead to changes in the hydroxyl groups. The peaks in the range of 3000 - 2800 cm^{-1} indicate CH bound both with stretching vibration. The wave number between 1700 and 1600 cm^{-1} indicates carbanyl group (C=O) stretching vibration (Ma et al., 2017). The peaks between 1440 and 1395 cm^{-1} could be the C-O-H of carboxylic acid (Kong et al., 2015). The peaks in the range of 1400 - 1380 cm^{-1} indicate methyl groups (CH_3) with symmetrical bending vibration and C-O stretching of carboxylic acids.

DOMP-NP presented peaks at 1074 cm^{-1} and 1235 cm^{-1}, which indicated that the

unsaturated ether (=C-O-C) was not shown on any DOMP with dual enzymatic hydrolysis. The bands between 1200 and 1000 cm^{-1} may result from alcohol C-OH groups as well as β-1,4 glucoside and β-1,4 mannoside of glucomannan with the C-O-C stretch vibration (Yang et al., 2015). Additionally, β-D-glucose pyranose, β-D-galactose and mannose had absorptive peaks at 900 - 870 cm^{-1}, 876 - 830 cm^{-1}, and 800 cm^{-1}, respectively. The FTIR results indicate a structural change with enzymatic hydrolysis.

3.3.2. SEM

Fig. 3-left shows the surface morphology of DOMP samples with enzymatic hydrolysis after lyophilisation. Previous studies suggest that the structures, properties and surface morphology of polysaccharides could be affected by the extraction, purification, and preparation conditions (Nep & Conway, 2010). DOMP samples treated with enzymes showed different shapes with various particle sizes. DOMP-NP presented aggregations of spherical particles, and DOMP-Amase showed fibre and branching layers. DOMP-Mase, DOMP-Gase, DOMP-Arase, DOMP-Xase and DOMP-Rase showed different spherical particle sizes with various conjugations.

Freshly prepared solutions of hydrolysed DOMP samples were dropped and dried on the prepared carbon-coated copper sheet, and the surface appearances were observed and are shown in Fig. 3-right. All DOMP samples treated with enzymes had the shape of a sphere at different sizes. DOMP-NP presents a relatively uniform sphere shape with aggregations, and the diameter of DOMP-NP was approximately 51.56 nm. Compared to DOMP-NP, DOMP-Mase and DOMP-Arase showed smaller

particles with diameters of 35.16 nm and 48.05 nm respectively. DOMP-Amase, DOMP-Gase, DOMP-Xase and DOMP-Rase showed different particle sizes that illustrated that some particles were flocculated to larger particles. The different particle sizes of DOMP-Amase, DOM-Xase, and DOMP-Rase were approximately 42.19～145.46 nm, 44.53～127.27 nm, and 31.64～81.81 nm, respectively. DOMP with both protease and galactanase treatment obtained diameters of 36.33～109.09 nm particles and were tightly aggregated. Therefore, both appearances of hydrolysed DOMP samples with freeze-drying and dried DOMP solution samples with enzymatic hydrolysis indicate that enzyme hydrolysis could change the structures of polysaccharides, reduce molecular weight, and debranch the lateral chains of polysaccharides.

3.3.3. Particle sizes of DOMP with enzymatic hydrolysis

The particle sizes (μm), dispersity index (PDI) and zeta potential values (mV) of enzymatically hydrolysed DOMP solutions (0.8% w/v) are shown in Table 3. The diameter of DOMP-NP was approximately 0.87 μm, which was significantly lower than the particle sizes of DOMP treated with dual enzyme hydrolysis. The particle sizes were consistent with the results shown in Fig. 3-right, which revealed that enzymatic hydrolysis could reduce the particle sizes. The results suggest that protease hydrolysed the glycoprotein, and dual enzyme hydrolysis debranched the lateral chain of polysaccharides due to the reaction of glycan hydrolase. Therefore, dual enzymatically hydrolysed DOMP samples contained larger and inconsistent droplet sizes. Since the DOMP solutions were presented acidic (pH values were shown in

Table 1), the zeta-potential values are negative. Zeta-potential values of enzymatically hydrolysed DOMP samples were from -24 to -18 and were not close enough to |±30|.

3.4. Viscosity of DOMP with enzymatic hydrolysis

The dependence of shear viscosity (η) was tested at 25 ℃ for shear rates in the range of 0.1 to 100 s^{-1}. With increasing shear rate, the viscosities of hydrolysed DOMP sample solutions (0.8% w/v) were maintained (data not shown). Therefore, DOMP with enzymatic hydrolysis at 0.8% w/v appeared to have Newtonian properties, and the viscosities are listed in Table 3. The viscosity of DOMP-NP was 1.94×10^{-3} Pa·s, which was significantly higher compared to the other DOMP with dual enzyme hydrolysis. The lowest viscosity is DOMP-Arase, which was approximately 1.23×10^{-3} Pa·s. The viscosity and molecular weight of DOMP samples were as follows in descending order: DOMP-Xase > DOMP-NP > DOMP-Gase > DOMP-Rase > DOMP-Amase > DOMP-Mase > DOMP-Arase, and DOMP-NP > DOMP-Xase > DOMP-Rase > DOMP-Arase > DOMP-Gase > DOMP-Amase > DOMP-Mase, respectively. The molecular weight of DOMP-NP and DOMP-Xase were approximately 69.5 kDa and 67.7 kDa (Table 2), respectively, and the viscosities of both samples were highest with no significant difference.

According to Whistler & Daniel (1990), the viscosity increased with the increase of molecular weight. The viscosity of a solution with highly branched structure is generally lower than linear molecules at the same molecular weight, because the linear molecules require more space for gyration than highly branched or bush-shaped molecules of the same molecular weight (Whistler & Daniel, 1990). Therefore,

DOMP-NP and DOMP-Xase presented higher viscosity due to the larger molecular weight. The similar molecular weight (approximately 67 kDa) of DOMP-Arase, DOMP-Xase and DOMP-Rase presented the significantly different viscosities, 1.23 × 10^{-3} Pa·s, 1.99 × 10^{-3} Pa·s and 1.63 × 10^{-3} Pa·s, respectively. The results implied that the DOMP-Xase may contain more linear structures or a few debranched polysaccharide chains. Meanwhile, DOMP-Arase may obtain more branched polysaccharides.

4. Conclusions

This study investigated the influence of enzymatic hydrolysis on the characterisation of *Dioscorea opposita* mucilage polysaccharides. The results help to characterise the relationship between functions and structures of DOMP. Enzymatic hydrolysis could reduce the molecular weight and consequently decrease the viscosity, yet increase the particle sizes. The results suggest that enzymatic degradation changed the structure of polysaccharides and led to physicochemical characterisation changes. DOMP contained 62.52% mannose and 23.45% glucose. The content of mannose in DOMP was decreased severely after protease hydrolysis (from 62.52% to 3.96%), which indicated that the mannose may be served as the structural skeleton of plant cell, and additionally, the protein and mannose may interact with each other. In this way, enzymatically hydrolysed DOMP not only helped to reveal the structure of mucilage polysaccharide from *Dioscorea opposita*, but also contributed to generating food by-products with specific requirements.

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Table 1. Yield, protein content, and monosaccharide compositions of DOMP with various enzymatic modification.

Sample Code	DOMP	DOMP-NP	DOMP-Amase	DOMP-Mase	DOMP-Gase	DOMP-Arase	DOMP-Xase	DOMP-Rase
Modification treatment	None	Protease	Protease + α-amylase	Protease + mannanase	Protease + galactanase	Protease + arabinase	Protease + xylanase	Protease + rhamnase
Yield (%)	5.71 ± 0.59	3.61 ± 0.35	3.88 ± 0.37	4.15 ± 0.21	4.43 ± 0.29	4.46 ± 0.14	3.92 ± 0.30	3.40 ± 0.37
Protein Content (%)	13.39 ± 0.49	4.62 ± 0.54	0.06 ± 0.002	2.18 ± 0.04	3.77 ± 0.54	1.51 ± 0.01	0.99 ± 0.17	1.10 ± 0.06
pH	6.58 ± 0.07	5.36 ± 0.02	5.91 ± 0.06	5.85 ± 0.08	5.90 ± 0.07	5.62 ± 0.05	5.21 ± 0.08	5.73 ± 0.07
Monosaccharides (%)								
Arabinose	3.33	5.38	18.06	21.96	26.52	17.52	18.45	25.19
Galactose	0.35	19.64	30.69	31.14	41.16	49.63	27.00	35.89
Glucose	23.45	45.10	10.51	19.03	7.44	9.75	12.26	11.08
Mannose	62.52	3.96	31.71	22.09	18.18	17.01	35.99	21.06
Rhamnose	0.42	2.51	2.44	3.05	3.28	3.97	3.15	3.55
Ribose	0.07	0.14	0.04	ND	0.04	0.05	0.05	0.07
Xylose	0.42	0.79	0.74	ND	ND	0.92	0.67	1.29
Galacturonic acid	0.01	22.11	2.90	0.37	0.13	ND	0.22	0.27
Glucuronic acid	0.02	0.38	2.90	2.36	3.25	1.14	2.21	1.60

Note: ND = None detected; detection limits for ribose, xylose and galacturonic acid were 48.64 μg/g, 27.29 μg/g, and 38.32 μg/g.

Table 2. Molecular weight distribution of DOMP with various enzymatic modifications.

Sample Code	Molecular weight (MW, Daltons) and PDI (Mw/Mn) in parentheses	Molecular Weight Distributions (Daltons)					
		< 3,000	3,000-10,000	10,000-20,000	20,000-100,000	100,000-200,000	> 200,000
DOMP-NP	69,483 (1.896)	0.00	4.50	10.50	63.25	18.75	3.00
DOMP-Amase	64,315 (1.801)	0.00	4.00	11.50	66.00	17.25	1.25
DOMP-Mase	63,923 (2.136)	0.00	6.75	14.50	60.25	15.50	3.00
DOMP-Gase	65,122 (1.693)	0.00	2.00	11.50	69.00	16.25	1.25
DOMP-Arase	67,280 (2.160)	0.00	4.00	15.75	63.75	14.50	2.00
DOMP-Xase	67,700 (2.003)	0.00	5.00	11.75	63.25	16.00	4.00
DOMP-Rase	67,685 (1.858)	0.00	4.50	9.50	66.00	18.00	2.00

Table 3. Viscosity, particles sizes (diameters, μm) and zeta-potential (mV) of the solution of modified DOMP samples (0.8% w/v, 25 °C)

	Viscosity (× 10^{-3} Pa·s)	Particle sizes (μm)	Mean PDI	Zeta-potential (mV)
DOMP-NP	1.94 ± 0.03[a]	0.87 ± 0.06[c]	0.14	-19.70 ± 0.26
DOMP-Amase	1.48 ± 0.05[b]	0.99 ± 0.07[c]	0.33	-22.90 ± 0.36
DOMP-Mase	1.42 ± 0.07[b]	1.17 ± 0.02[d]	0.36	-18.30 ± 1.00
DOMP-Gase	1.84 ± 0.07	1.84 ± 0.08[e]	0.42	-20.50 ± 0.26
DOMP-Arase	1.23 ± 0.05	1.73 ± 0.09[e]	0.40	-18.30 ± 0.87
DOMP-Xase	1.99 ± 0.07[a]	1.12 ± 0.11[d]	0.32	-20.00 ± 0.42
DOMP-Rase	1.63 ± 0.04	1.66 ± 0.04	0.34	-24.70 ± 0.76

Note: Results are presented as the mean ± standard deviation; Paired values with superscript letters a to e indicate no significant difference ($P > 0.05$).

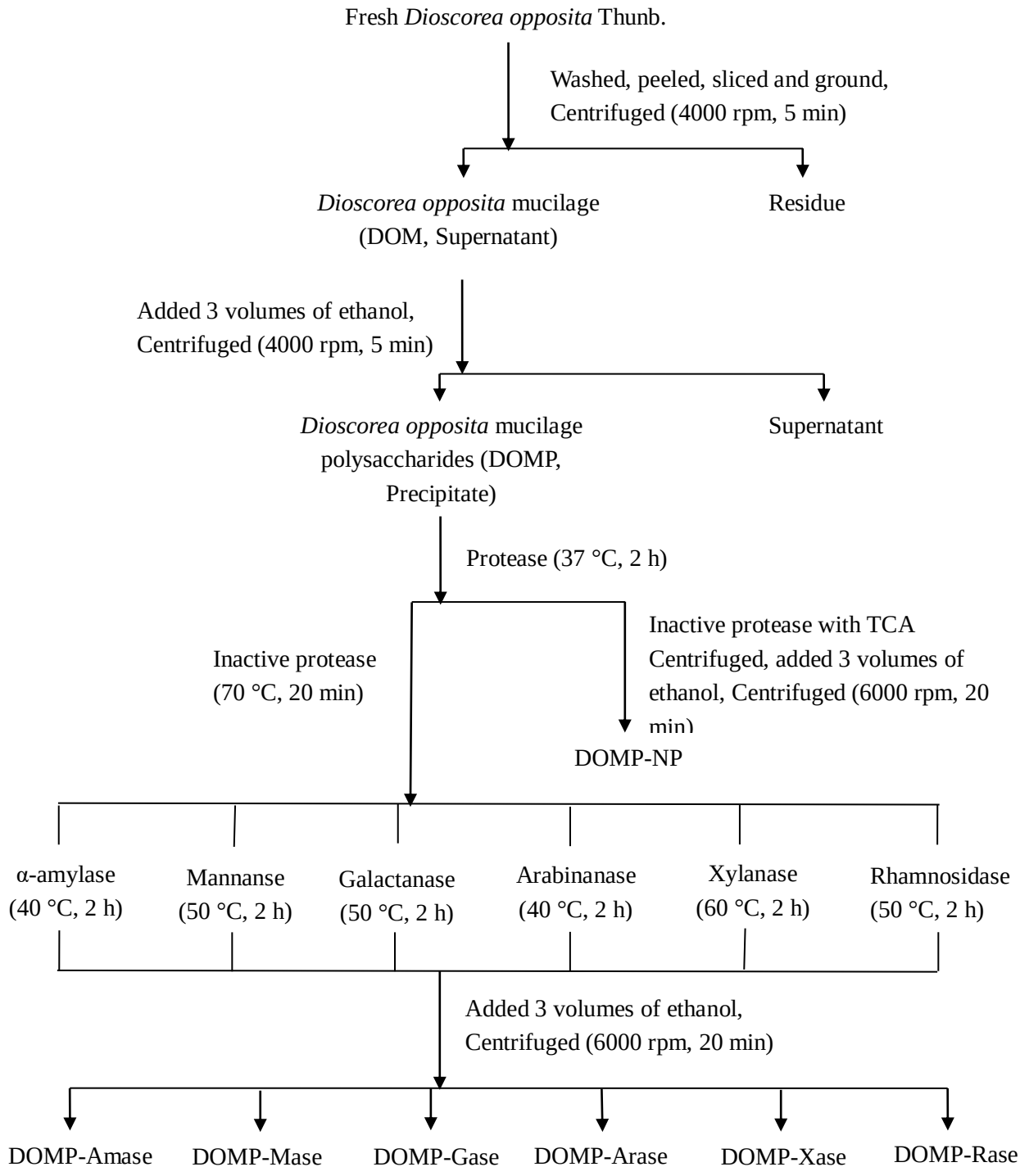


Fig. 1. Flow chart of enzymatic modifications of *Dioscorea opposita* mucilage polysaccharides (DOMP)

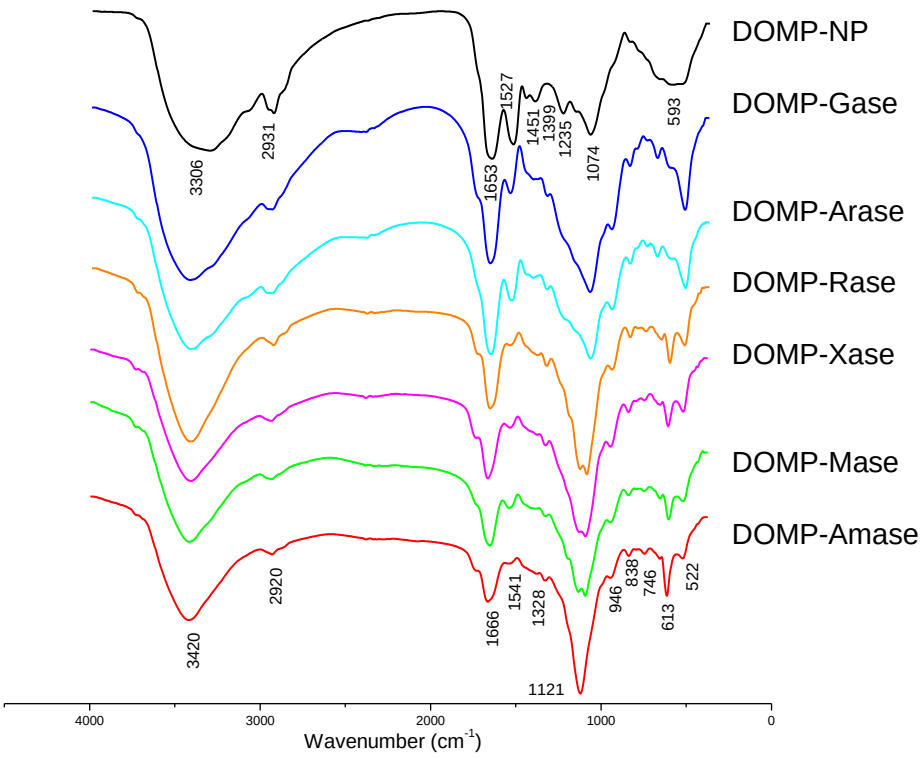
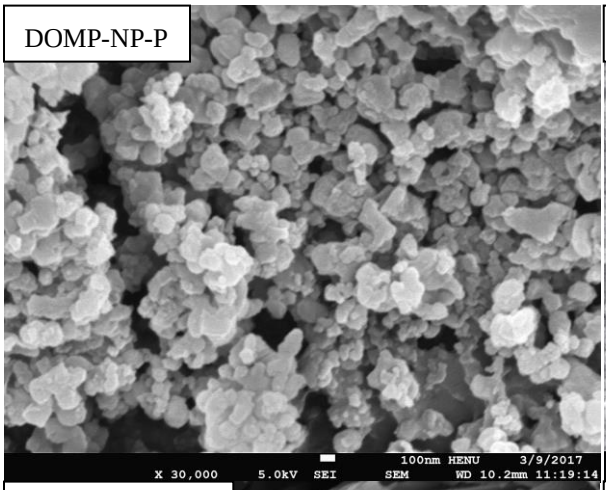
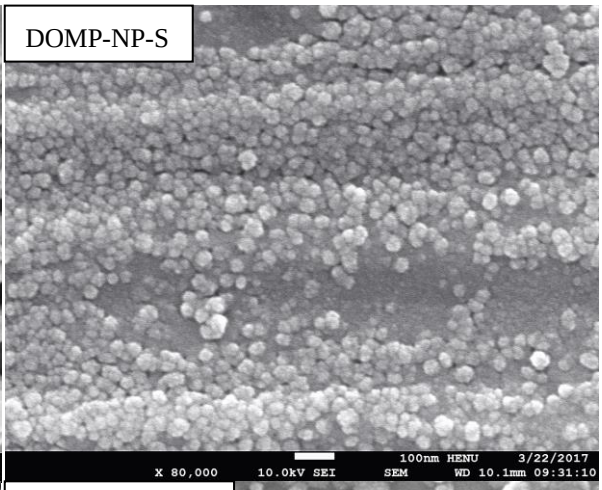
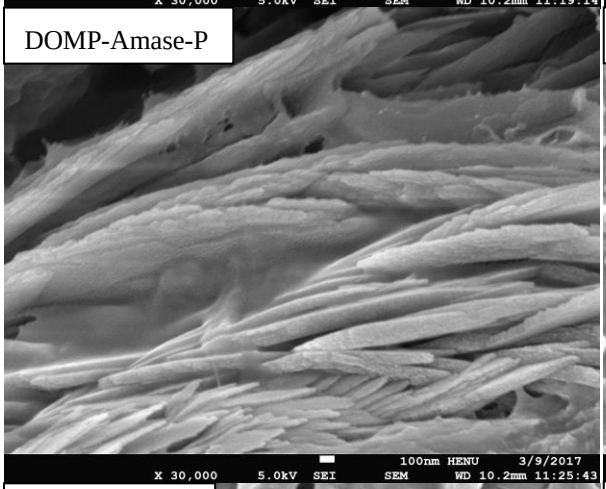
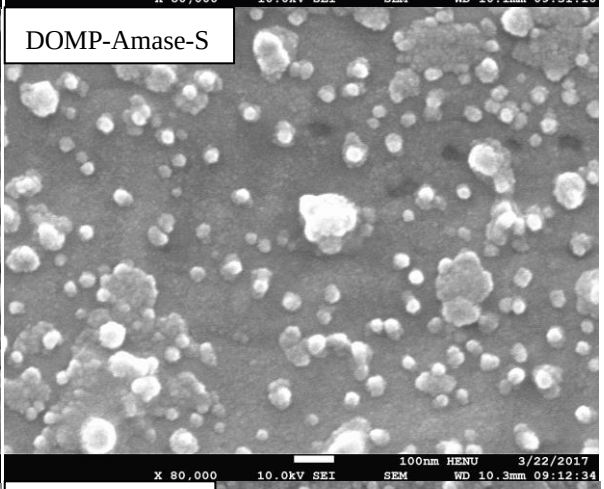


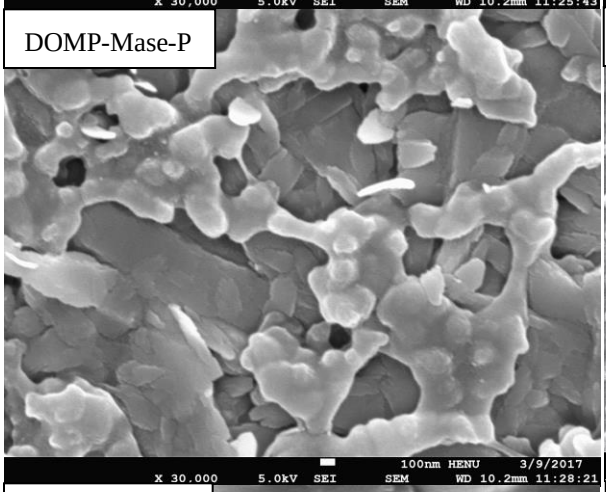
Fig. 2. FT-IR spectrums of DOMP samples with enzymatic modification

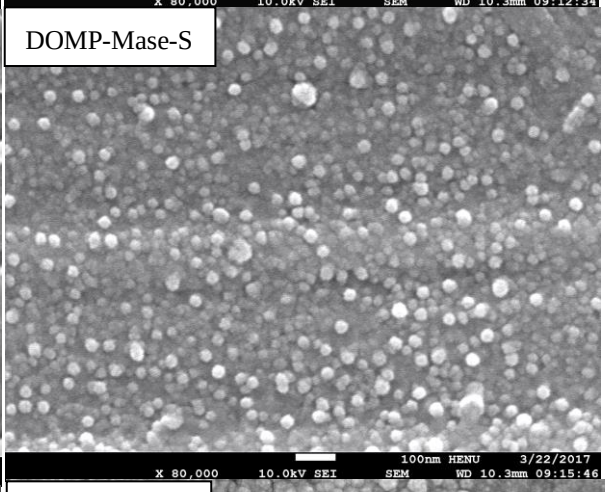

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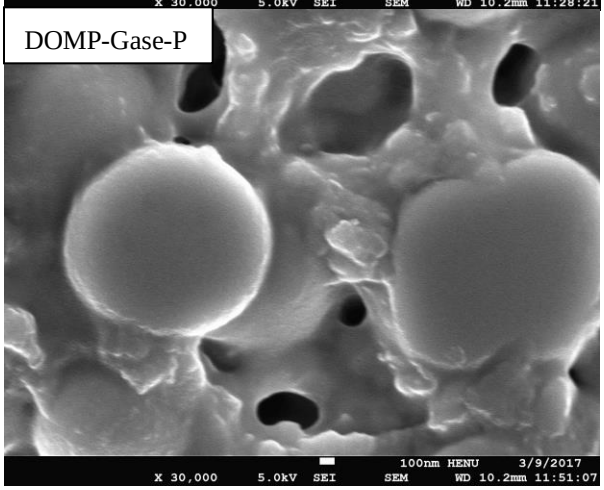

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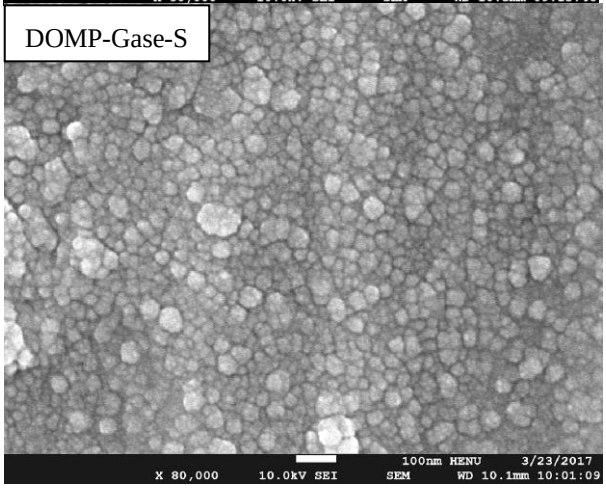

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100nm HENU 3/9/2017


DOMP-Amase-S
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100nm HENU 3/22/2017


DOMP-Mase-P
X 30,000 5.0kV SEI SEM WD 10.2mm 11:28:21
100nm HENU 3/9/2017


DOMP-Mase-S
X 80,000 10.0kV SEI SEM WD 10.3mm 09:15:46
100nm HENU 3/22/2017


DOMP-Gase-P
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100nm HENU 3/23/2017

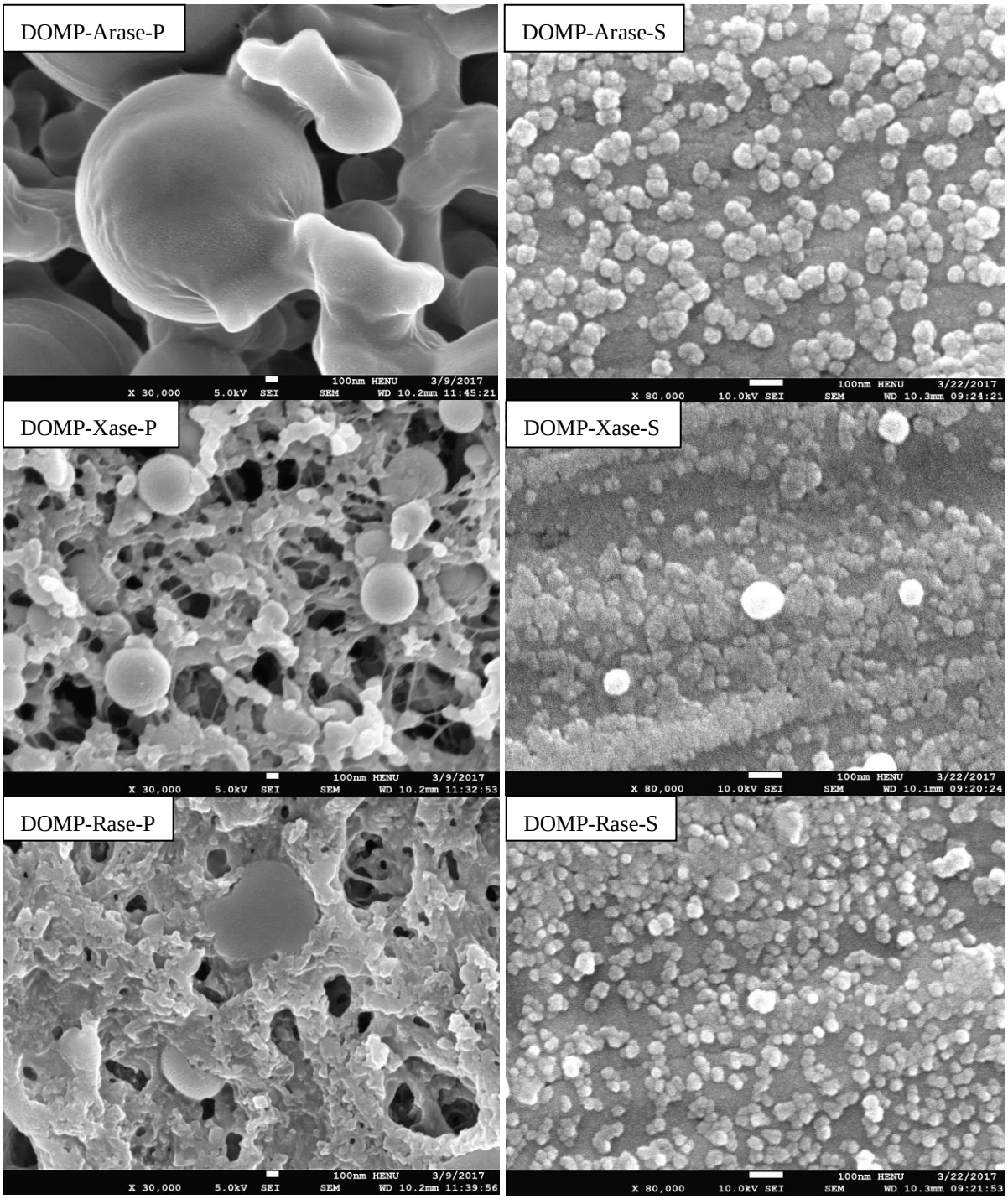


Fig. 3. Scanning electron microscopic images of enzymatically modified DOMP after freeze-drying (left) and surface morphology of modified DOMP dried solutions (right), at magnifications of ×30,000 and ×80,000, respectively.