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

Running Title: Enzymatic hydrolysis of yam mucilage polysaccharides

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28 ## Abstract

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

43

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

45

46 **Abbreviations:**

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

49

50 ## 1. Introduction

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

61 *Dioscorea opposita* Thunb., the Chinese yam (CY), is a tuber crop that has
62 nutritional and economic significance in China (Zhang, et al., 2014). According to
63 previous studies, *Dioscorea opposita*, which is an important edible and
64 pharmaceutical food in China, contains various chemical components and nutrients,
65 including polysaccharides, amino acids, flavonoids, allantoin, dopamine, and
66 batatasin (Chen et al., 2015; Yang et al., 2008; Wang et al., 2006). *Dioscorea opposita*
67 has bioactivity and health benefits, such as enhancing immunity, lowering blood sugar,
68 and has pharmacological functions, including treating haemorrhoids, sore throat and
69 struma, lung diseases and the pancreas disease, etc. (Chan & Ng, 2013; Ma et al.,
70 2017).

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

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

78 Currently, enzymatic hydrolysis has been used to improve or customise the
79 properties as well as modify the structures of existing polysaccharides (Cheng & Gu,
80 2012; Zeng & Lai, 2016). Kim et al. (2013, 2014) reported that structural
81 modification by enzymes changed the physical behaviour of their model pectin.
82 Enzymatic hydrolysis also lowers the molecular weight or debranches the lateral
83 chains of polysaccharides, which could lead to valuable polysaccharide applications
84 (Leathers et al., 2015). Jo et al. (2016) investigated the nutritional quality and the
85 development of new dietary applications of sweet potato as well as value-added
86 products generated though enzymatic modification of starch. Despite the relatively
87 low yields from enzymatic reactions, modified polysaccharides with a lower
88 molecular weight still maintain their desired end-use properties (Cheng & Gu, 2012;
89 Zeng & Lai, 2016).

90 The mucilage of *Dioscorea opposita* (DOM) was comprised of protein (≈ 2.78%),
91 and polysaccharides, including glucose (≈ 49.50%), mannose (≈ 33.40%), galactose
92 (≈ 10.90%), xylose (≈ 5.38%), arabinose (≈ 0.54%), and rhamnose (≈ 0.25%). The
93 molecular weight (MW) of DOM was 143,700 Da (Ma et al., 2017). This study was

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

100 2. Materials and Methods

101 2.1. Materials

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

114 2.2. Extraction and enzymatic treatment of *Dioscorea opposita* mucilage
115 **polysaccharide (DOMP)**

116 2.2.1. Extraction of DOMP

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

127 2.2.2. Preparation of DOMP samples with enzymatic hydrolysis

128 Enzymatic hydrolysis of DOMP was carried out according to the methods
129 described by Zeng and Lai (2016) with modifications. DOMP was divided into two
130 separated portions for various enzymatic hydrolysis procedures (flow chart shown in
131 Fig. 1). The first portions of DOMP were used for protease hydrolysis. 4.00 mg
132 DOMP were dissolved with 125 mL of 50 mM phosphate buffer (pH 7.0), followed
133 by adding approximately 50 U of protease and incubating the solution at 37 °C for 2 h.
134 25.0 mL of 9.0% trichloroacetic acid (TCA) were then added to terminate the
135 proteinase reaction. The mixture was subsequently centrifuged (6,000 rpm, 20 min),
136 and the supernatant was dialysed against deionised water by using a dialysis
137 membrane (MWCO, 500 Da, Solarbio Life Sciences, Beijing, China). Three volumes

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

141 Another portion of DOMP was carried out for dual enzymatic hydrolysis
142 procedures. The same protease hydrolysis procedure was performed as described
143 previously, except that the proteinase treatment was terminated by heating at 70 °C for
144 20 min. After cooling, 108.9 U of α-amylase (Amase), 58.4 U of mannanase (Mase),
145 94.2 U of galactanase (Gase), 45.6 U of xylanase (Xase), 21.8 U of arabinanase
146 (Arase), or 30.4 U of rhamnosidase (Rase) were added separately and incubated at
147 40 °C, 50 °C, 50 °C, 40 °C, 60 °C, and 50 °C, respectively, for 2 h. The reaction
148 mixtures were then centrifuged, dialysed, precipitated with ethanol, and lyophilised as
149 previously described to obtain the deproteinised DOMP with Amase (DOMP-Amase),
150 Mase (DOPM-Mase), Gase (DOMP-Gase), Xase (DOMP-Xase), Arase
151 (DOMP-Arase), or Rase (DOMP-Rase) hydrolysis, respectively. The samples were
152 stored in vacuum desiccators over phosphorus pentoxide until they were used.

153 **2.3 Characterisation of DOMP with enzymatic hydrolysis**

154 2.3.1. Yield

155 4.00 mg of DOMP were used each time to modify the structure, and the final
156 hydrolysed DOMP was lyophilised and weighed. Therefore, the yield (%) of
157 enzymatically hydrolysed DOMP was calculated by the following formula:

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

158

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) derivativation 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 $mol \cdot L^{-1}$ 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 $1.0\ mL \cdot min^{-1}$. 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

181 measured using high-performance size-exclusion chromatography

182 (HPSEC-MALLS-RID, Wyatt Technology Co., USA) with an OHpak SB-802.5 HQ

183 column (8.0 mm × 300 mm, Shodex Co., Japan). The mobile phase was 0.1 M $NaNO_3$

184 at a flow rate of 0.5 $mL·min^{-1}$, 50.0 μL of sample solutions (1.8 $mg·mL^{-1}$) were

185 injected, and the chromatogram was analysed using ARTRAV software (Wyatt

186 Technology Co., USA).

187 2.3.6. Fourier transform infrared spectroscopy (FT-IR)

188 Enzymatically hydrolysed DOMP samples were analysed using FT-IR (Vertex 70,

189 Bruker, Germany) with a spectral range of 4000 to 400 cm^{-1}. The transmission of the

190 samples within 7 mm diameter KBr pellets was measured.

191 2.3.7. Scanning electron microscopy (SEM)

192 The hydrolysed DOMP samples were taken after freeze-drying and prepared by

193 sticking them to one side of double-sided adhesive tape attached to a circular

194 specimen stub, and sputter coated with vacuum spray gold. Moreover, freshly

195 prepared solutions of hydrolysed DOMP samples were diluted, dropped on the

196 prepared carbon-coated copper sheet and left to dry at room temperature (20 °C). The

197 samples were completely dried and sputter coated with vacuum spray gold. A thermal

198 field emission scanning electron microscope (JSM-7001F, JEOL Ltd., Japan) was

199 used to inspect the morphology of enzymatically hydrolysed DOMP samples.

200 2.3.8. Particle sizes

201 The droplet diameters and zeta-potential of the solutions made by hydrolysed

202 DOMP samples were investigated using Malvern zeta-potential (Malvern-NanoZS90,

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

206 2.3.9. Viscosity

207 The viscosity of hydrolysed DOMP samples was measured by rotatory rheometer
208 (TA-DHR2, TA Instruments, New Castle, Delaware, USA) with a 60 mm cone plate
209 (2°). Flow sweep measurements were carried out to determine the viscosity with a
210 shear rate in the range of 0.01 s^{-1} to 100 s^{-1}. Samples were loaded onto the rheometer,
211 and it was allowed to equilibrate to the measuring temperature (25 ± 1 °C, ≈ 0.5 min).
212 For each test, approximately 2 mL samples were transferred onto the plate.

213 3. Results and Discussion

214 3.1. Yield and chemical compositions of DOMP with enzymatic hydrolysis

215 The yield of *Dioscorea opposita* mucilage (DOM) and *Dioscorea opposita*
216 mucilage polysaccharides (DOMP) were approximately 8.18% and 5.70%,
217 respectively (Ma et al., 2017). Enzymatic hydrolysis treatment significantly reduced
218 the yield of DOMP samples as expected (in the range of 3.40% to 4.46%, shown in
219 Table 1). The yield of DOMP samples treated by protease alone was 3.61%, which
220 was lower than other DOMP samples treated by protease + α-amylase (DOMP-Amase,
221 3.88%), protease + mannanase (DOMP-Mase, 4.15%), protease + galactanase
222 (DOMP-Gase, 4.43%), protease + arabinase (DOMP-Arase, 4.46%), and protease +
223 xylanase (DOMP-Xase, 3.92%). The results indicate that protein may interact with
224 both the large and small polysaccharide fractions of DOMP and precipitate after

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

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

238 The main monosaccharides in DOMP were 62.52% mannose, 23.45% glucose,
239 9.30% xylose, and 3.33% arabinose. Single protease-treated DOMP terminated by
240 TCA contained 45.10% glucose, 22.1% galacturonic acid, 19.64% galactose, 5.38%
241 arabinose, and 3.96% mannose. Interestingly, the biggest difference is in the mannose
242 content. The mannose contents in DOMP and DOMP-NP were 62.52% and 3.96%,
243 respectively, which indicates that most mannose in the mucilage of *Dioscorea*
244 *opposita* is more likely to be straight chains and serve as the structural skeleton of
245 plant cells (Coultate, 2002). Schmitt et al. (2009) stated that protein and
246 polysaccharides can be found in the same physiological environment and interact. The

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

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

269 monosaccharide contents in samples treated with high temperature inactivation.

270 3.2 Molecular weight (MW) and MW distributions of DOMP with enzymatic 271 hydrolysis

272 The molecular weight, polydispersity (PDI, Mw/Mn), and distribution details are 273 shown in Table 2. The molecular weight (MW) of DOMP-NP was 69,483 Daltons, 274 higher than the rest of the dual enzymatically hydrolysed DOMP samples. The 275 decrease in molecular weight implied that the protein might integrate with 276 polysaccharides, and polysaccharides were partially removed from the structures 277 (Zeng et al., 2016). Although dual enzymatic hydrolysis through the action of 278 proteinase and monosaccharidase decreased the molecular weight of DOMP, the 279 pattern of molecular weight distribution was intact.

280 The molecular weight was distributed into six sections, < 3, 3-10, 10-20, 20-100, 281 100-200, and > 200 kDa, and it was mainly in the range of 20-100 kDa. The 282 molecular weight distributions of DOMP-Amase, DOMP-Gase, and DOMP-Rase 283 were approximately 66.00%, 69.00%, and 66.00% respectively in the range of 20 to 284 100 kDa, which were higher than molecular weight in 20-100 kD of DOMP-NP 285 (63.25%). Particularly, DOMP treated with both protease and mannanase had a higher 286 yield (4.15%), lower molecular weight (63,923 Dalton), and a relatively low amount 287 in the range of 20-100 kDa, which suggested that the proteinase cleaved the bound 288 protein from polysaccharides, and smaller molecular weight of polysaccharides were 289 precipitated. Interestingly, DOMP-Gase had a high yield (4.43%), lower molecular 290 weight (65,122 Dalton), and 69.00% was in the range of 20-100 kDa. The MW

291 distribution of DOMP-Gase was 2.00% in 3-10 kDa, 11.50% in 10-20 kDa, 69.00% in
292 20-100 kDa, 16.25% in 100-200 kDa, and 1.25% were larger than 200 kDa, which
293 demonstrated that the MW distribution was concentrated to 20-100 kDa. The results
294 suggest that proteins in DOMP were hydrolysed, which led to two possibilities: first,
295 some smaller molecular polysaccharides may co-precipitate from the addition of TCA
296 due to the changes in pH and temperature, and second, proteinases may break the
297 linkage of proteins and polysaccharides, and those proteins or polysaccharides were
298 rearranged and aggregated (Zeng et al., 2016).

299 **3.3. Characterisation of DOMP with enzymatic hydrolysis**

300 3.3.1. FTIR

301 Fig. 2 shows the FTIR for enzymatically hydrolysed DOMP samples. The wide
302 bands in 3700 - 3000 cm^{-1} indicate hydroxyl groups (-OH) (Andrade et al., 2015).
303 DOMP with protease treatment presents the peak at 3306 cm^{-1}, which moved to 3420
304 cm^{-1} with dual enzymatic treatment and implied that dual enzymes with their optimal
305 pH lead to changes in the hydroxyl groups. The peaks in the range of 3000 - 2800
306 cm^{-1} indicate CH bound both with stretching vibration. The wave number between
307 1700 and 1600 cm^{-1} indicates carbanyl group (C=O) stretching vibration (Ma et al.,
308 2017). The peaks between 1440 and 1395 cm^{-1} could be the C-O-H of carboxylic acid
309 (Kong et al., 2015). The peaks in the range of 1400 - 1380 cm^{-1} indicate methyl
310 groups (CH_3) with symmetrical bending vibration and C-O stretching of carboxylic
311 acids.

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

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

320 3.3.2. SEM

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

329 Freshly prepared solutions of hydrolysed DOMP samples were dropped and
330 dried on the prepared carbon-coated copper sheet, and the surface appearances were
331 observed and are shown in Fig. 3-right. All DOMP samples treated with enzymes had
332 the shape of a sphere at different sizes. DOMP-NP presents a relatively uniform
333 sphere shape with aggregations, and the diameter of DOMP-NP was approximately
334 51.56 nm. Compared to DOMP-NP, DOMP-Mase and DOMP-Arase showed smaller

335 particles with diameters of 35.16 nm and 48.05 nm respectively. DOMP-Amase,
336 DOMP-Gase, DOMP-Xase and DOMP-Rase showed different particle sizes that
337 illustrated that some particles were flocculated to larger particles. The different
338 particle sizes of DOMP-Amase, DOM-Xase, and DOMP-Rase were approximately
339 42.19～145.46 nm, 44.53～127.27 nm, and 31.64～81.81 nm, respectively. DOMP
340 with both protease and galactanase treatment obtained diameters of 36.33～109.09
341 nm particles and were tightly aggregated. Therefore, both appearances of hydrolysed
342 DOMP samples with freeze-drying and dried DOMP solution samples with enzymatic
343 hydrolysis indicate that enzyme hydrolysis could change the structures of
344 polysaccharides, reduce molecular weight, and debranch the lateral chains of
345 polysaccharides.

346 3.3.3. Particle sizes of DOMP with enzymatic hydrolysis

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

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

359 **3.4. Viscosity of DOMP with enzymatic hydrolysis**

360 The dependence of shear viscosity (η) was tested at 25 °C for shear rates in the
361 range of 0.1 to 100 s^{-1}. With increasing shear rate, the viscosities of hydrolysed
362 DOMP sample solutions (0.8% w/v) were maintained (data not shown). Therefore,
363 DOMP with enzymatic hydrolysis at 0.8% w/v appeared to have Newtonian
364 properties, and the viscosities are listed in Table 3. The viscosity of DOMP-NP was
365 1.94×10^{-3} Pa·s, which was significantly higher compared to the other DOMP with
366 dual enzyme hydrolysis. The lowest viscosity is DOMP-Arase, which was
367 approximately 1.23×10^{-3} Pa·s. The viscosity and molecular weight of DOMP
368 samples were as follows in descending order: DOMP-Xase > DOMP-NP >
369 DOMP-Gase > DOMP-Rase > DOMP-Amase > DOMP-Mase > DOMP-Arase, and
370 DOMP-NP > DOMP-Xase > DOMP-Rase > DOMP-Arase > DOMP-Gase >
371 DOMP-Amase > DOMP-Mase, respectively. The molecular weight of DOMP-NP and
372 DOMP-Xase were approximately 69.5 kDa and 67.7 kDa (Table 2), respectively, and
373 the viscosities of both samples were highest with no significant difference.

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

379 DOMP-NP and DOMP-Xase presented higher viscosity due to the larger molecular
380 weight. The similar molecular weight (approximately 67 kDa) of DOMP-Arase,
381 DOMP-Xase and DOMP-Rase presented the significantly different viscosities, $1.23 \times$
382 10^{-3} Pa·s, 1.99×10^{-3} Pa·s and 1.63×10^{-3} Pa·s, respectively. The results implied that
383 the DOMP-Xase may contain more linear structures or a few debranched
384 polysaccharide chains. Meanwhile, DOMP-Arase may obtain more branched
385 polysaccharides.

386 **4. Conclusions**

387 This study investigated the influence of enzymatic hydrolysis on the
388 characterisation of *Dioscorea opposita* mucilage polysaccharides. The results help to
389 characterise the relationship between functions and structures of DOMP. Enzymatic
390 hydrolysis could reduce the molecular weight and consequently decrease the viscosity,
391 yet increase the particle sizes. The results suggest that enzymatic degradation changed
392 the structure of polysaccharides and led to physicochemical characterisation changes.
393 DOMP contained 62.52% mannose and 23.45% glucose. The content of mannose in
394 DOMP was decreased severely after protease hydrolysis (from 62.52% to 3.96%),
395 which indicated that the mannose may be served as the structural skeleton of plant cell,
396 and additionally, the protein and mannose may interact with each other. In this way,
397 enzymatically hydrolysed DOMP not only helped to reveal the structure of mucilage
398 polysaccharide from *Dioscorea opposita*, but also contributed to generating food
399 by-products with specific requirements.
400

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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 ($\times 10^{-3}$ 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 ($P > 0.05$).

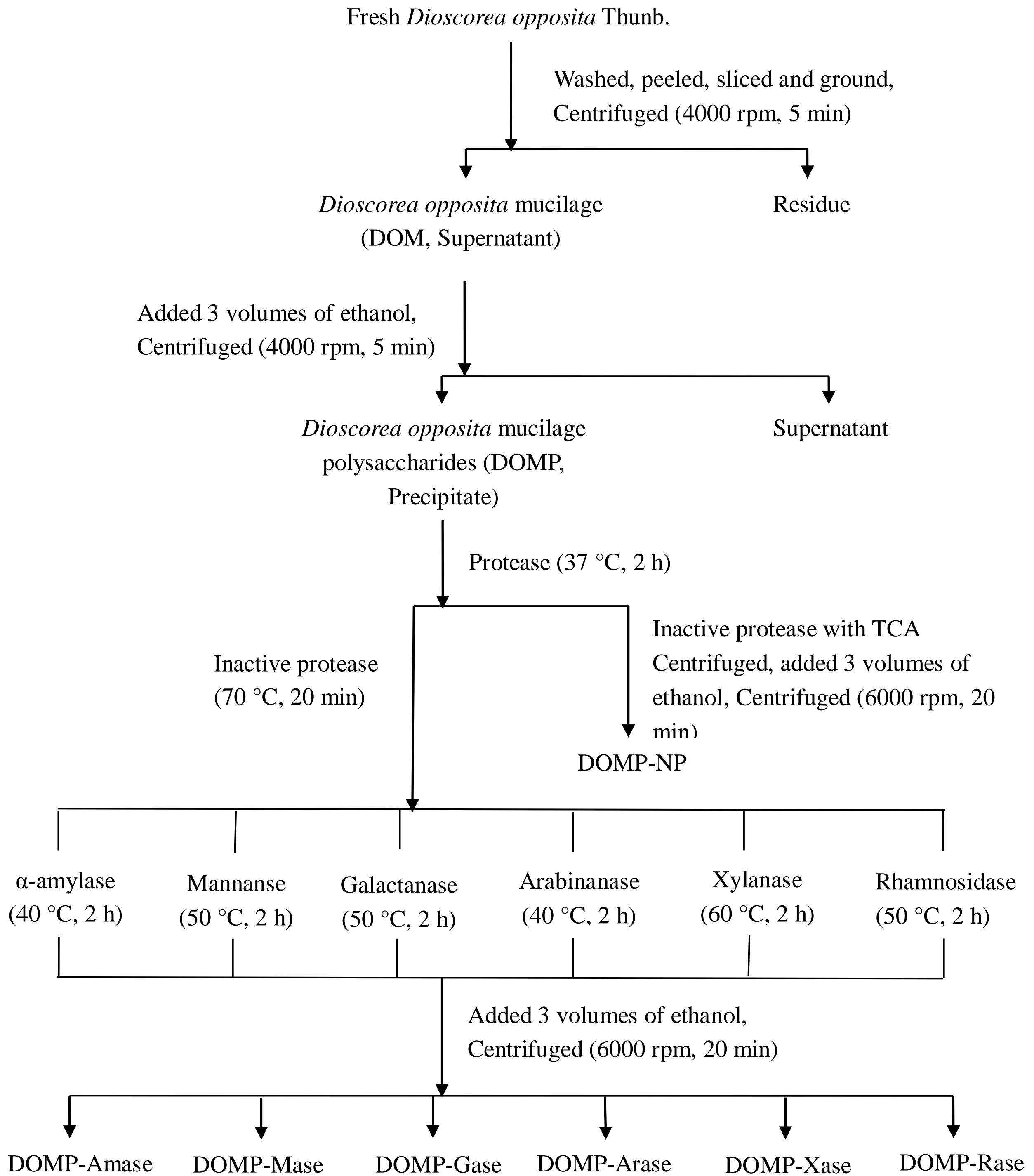


Fig. 1. Flow chart of enzymatic modifications of *Dioscorea opposita* mucilage polysaccharides (DOMP)

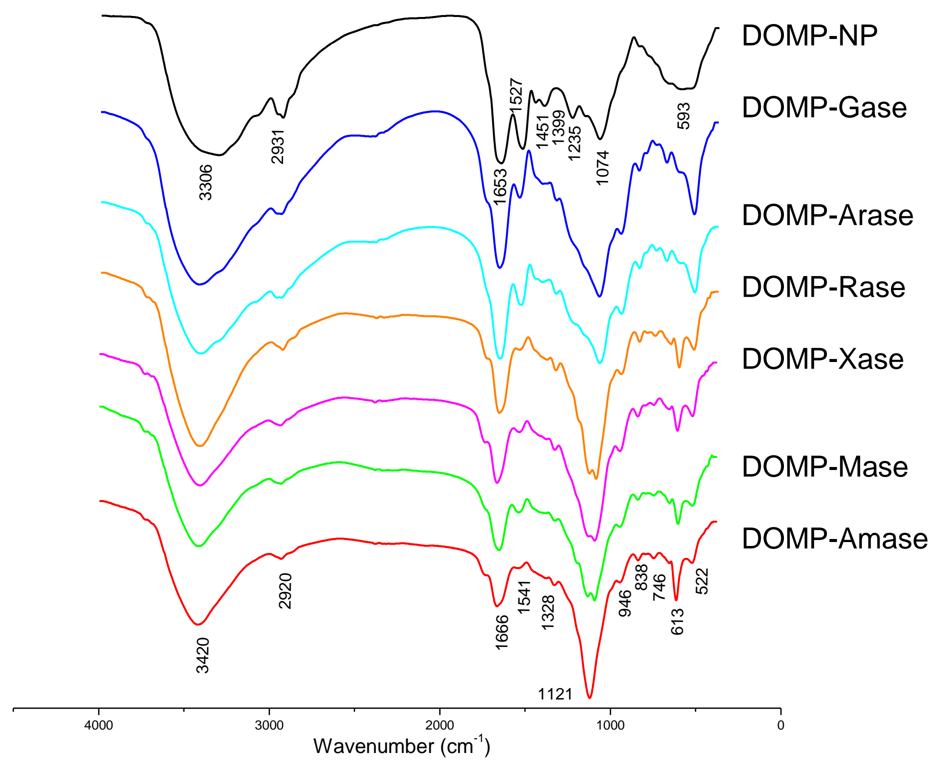
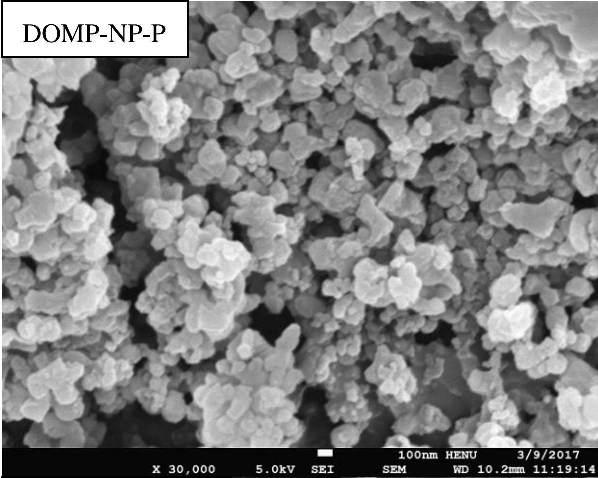


Fig. 2. FT-IR spectrums of DOMP samples with enzymatic modification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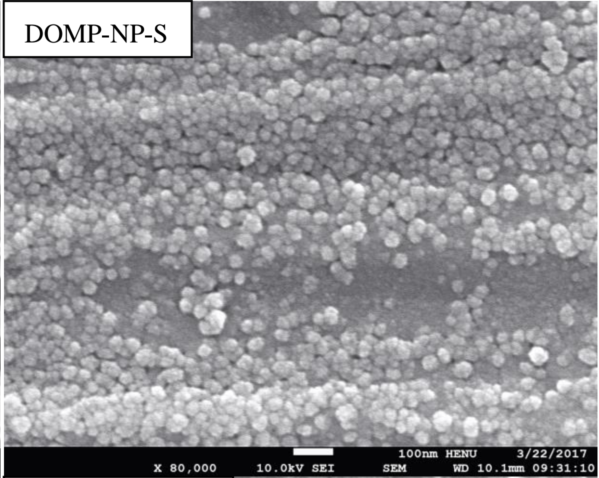
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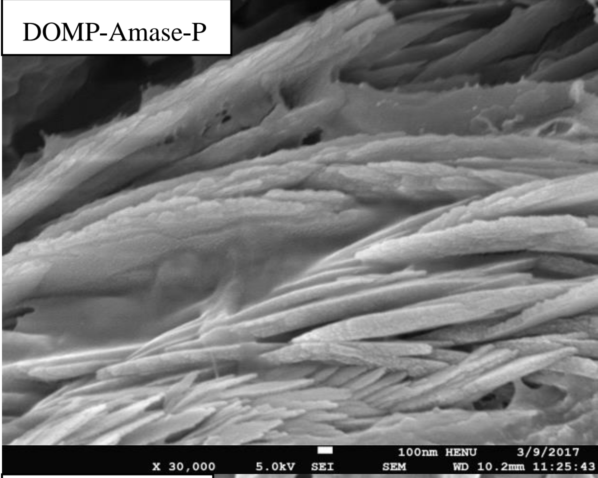
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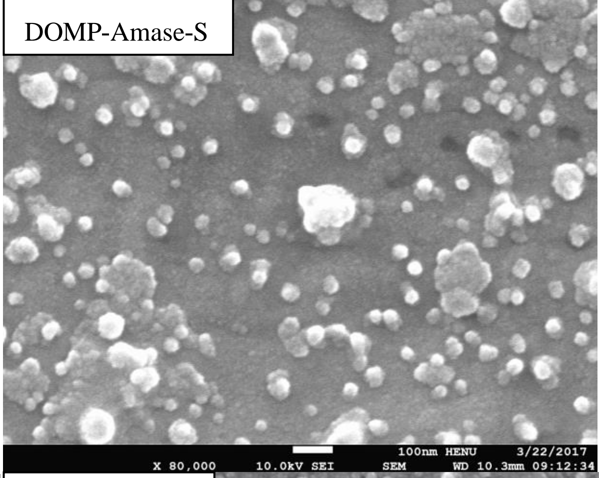
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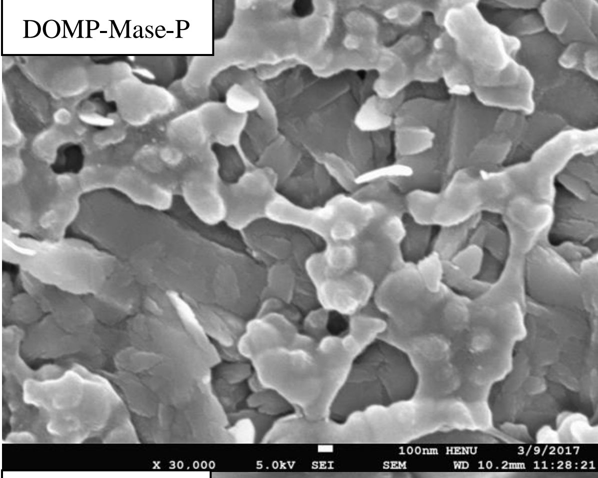
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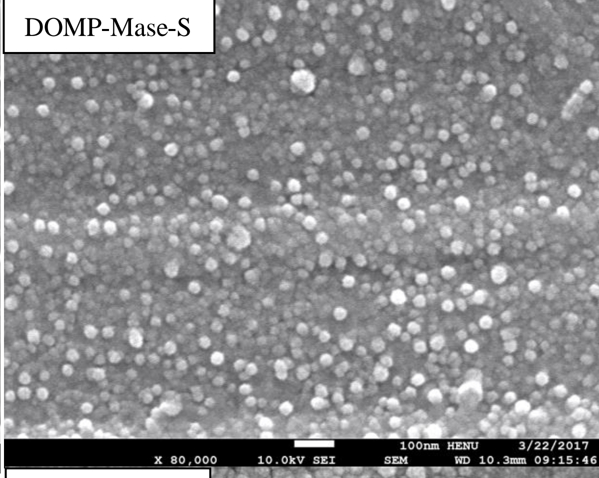
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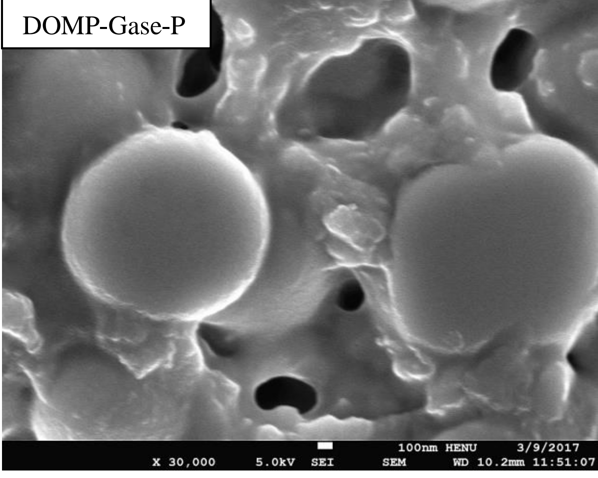
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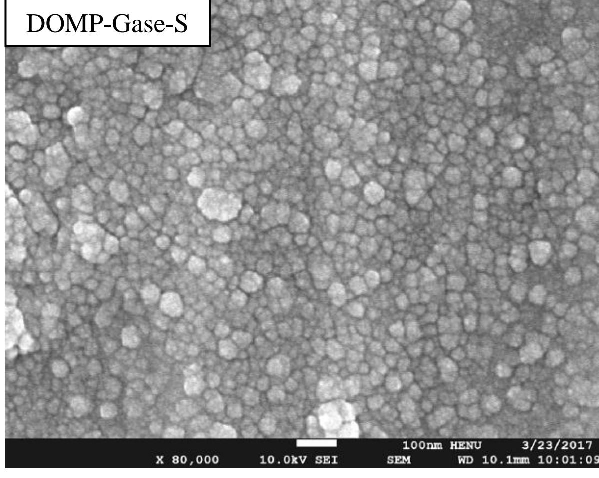
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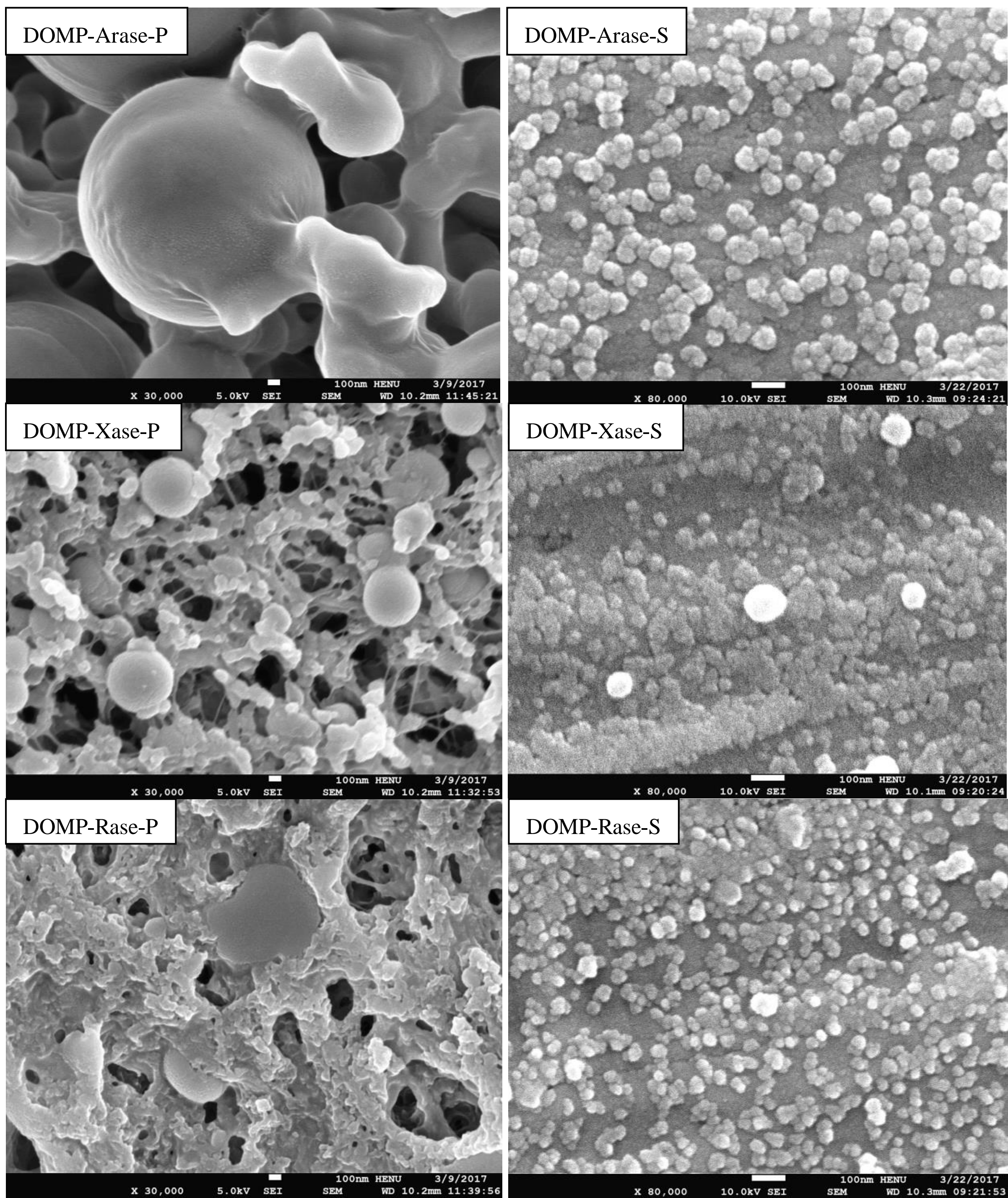

Fig. 3. Scanning electron microscopic images of enzymatically modified DOMP after freeze-drying (left) and surface morphology of modified DOMP dried solutions (right), at magnifications of ×30,000 and ×80,000, respectively.