Emulsification properties of polysaccharides from *Dioscorea opposita* Thunb.

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Abstract

This study investigated the emulsification properties of polysaccharides from *Dioscorea opposita* Thunb.. Graded alcohol precipitation was used to extract *Dioscorea opposita*polysaccharides fractions (4 samples) in different ranges of molecular weight. Sample 3 contained more glucose and protein (80.13% and 0.34%, respectively), and molecular weight was approximately 34,790 Da, distributing narrowly. The droplet sizes and stabilities of emulsions made ofgum arabic (GA) and polysaccharide samples at different concentrations and ratios were measured, specifically the emulsions of GA and medium-chain-triglycerides (MCT); polysaccharides and MCT; and polysaccharides, GA and MCT (1 : 1: 1). The results indicated that sample 2 and 3 had emulsifying properties, and the emulsions made with sample 2, GA and MCT (1 : 1 : 1) presented the best emulsification properties. Therefore, polysaccharides of *Dioscorea opposita* could be utilised as a natural emulsifier that can be improved synergistically with other emulsifiers, such as gum arabic.

Key Words: Chinese yam, *Dioscorea opposita* Thunb., polysaccharides, emulsification properties

Abbreviations:

CY	Chinese yam;	CYP	Chinese yam polysaccharides;
DOP	*Dioscorea opposita* Polysaccharides;		
GA	Gum arabic;	MCT	Medium-chain-triglycerides;
MW	Molecular weight;	Mw	Weight-average molecular weight;
PDI	Polydispersity index;	Mn	Number-average molecular weight;
S1, S2, S3 and S4	Sample 1, Sample 2, Sample 3 and Sample 4 of DOP;		

1. Introduction

Currently, there is considerable interest in using the food grade polysaccharides from natural plants in functional foods, dietary supplements, and health products (Harding et al., 2011). Various yam species of the genus *Dioscorea* have been widely used for health benefits in Asia for more than 2000 years. *Dioscorea opposita* Thunb., one type of Chinese yam (CY), is listed as both an edible plant and a traditional herbal medicine in China (Chang et al., 2004). *Dioscorea opposita* has been traditionally used to treat anorexia, chronic diarrhea, diabetes, seminal emission and excessive leucorrhea, as recorded in *SHEN NONG BEN CAO JING*, the earliest Chinese medicinal documents (Gao et al., 2007; Zheng et al., 2014; Zhang et al., 2011; Shi & Pan, 2010; Ye et al., 2010). The antioxidant, anti-inflammatory, neuro-protective and anti-cancer properties of Chinese yam polysaccharides (CYP) have been investigated to understand the scientific basis of their use as a functional food (Liu et al., 2008; Chan & Ng, 2013; Chiu et al., 2013; Son et al., 2014).

According to Zhang et al. (2016), the high molecular weight (MW) of CYP could seriously affect its food applications and functions. Thus, this study was performed to extract polysaccharides from *Dioscorea opposita* (DOP) by the gradedalcohol precipitation. Zhao et al. (2005) analysed the structures of Chinese yam polysaccharides (CYP) and determined the water-soluble polysaccharide was a heteropolysaccharide containing (1→3)-α-glucopyranose as a main chain and -β-galactopyranose-[(1→2)-α-Mannopyranose]$_3$-(1→2)-α-Mannopyranose-(1-6)- as a side chain. The MW was

42,000 Da. Yang et al. (2015) characterised structures of CYP and measured the MW as 16,619 Da. The differences in the MW of CYP reported in literature may be caused by species diversity from different locations and origins.

Nowadays, healthy and natural food products attracted concerns from consumers, who require food with better texture, taste, and other organoleptic properties (Li & Nie, 2016). Functional food products require scientific studies of dispersions, gels, and emulsions that can be organised and arranged in complex internal microstructures (Garti, 1999). Dickinson (2003) stated that one type of widely used hydrocolloid emulsifier in food applications is galactomannans. Protein emulsifiers are also traditionally excellent emulsifiers because they rapidly adsorb and rearrange molecular structures at the interface to provide a coherent macromolecular protective layer (Chanamai & McClements, 2002).

McClements (2005) illustrates that the droplet sizes and zeta-potential play an important role in determining the stability, appearance, texture and taste of the emulsions in the final product. Therefore, in order to control the properties of emulsions, it is required to obtain detailed quantitative information on the droplet size distribution on the changes occurring (Horne, 1995). Medium-chain-triglycerides (MCT) are used as a fat/lipid carrier to food flavours, essences, and pigments, which are widely used in food industry (Télessy et al., 2009). Hence, the droplet diameters and zeta-potential values of the oil/water (O/W) emulsions made by emulsifier with MCT were measured and analysed in this study.

Therefore, DOP could be recognised as an emulsifier in food due to its compositions of glucose, galactose and mannose as main monosaccharides and protein fractions. This study investigated the emulsification properties of DOP, with gum arabic (GA) as the control emulsifier. Gum arabic is one of the most extensively used exudate gums and a food hydrocolloid that displays both emulsifying and emulsion stabilising properties(Nakauma et al., 2008; Yadav et al., 2007; Ma et al., 2015).

2. Materials and Methods

2.1. Materials

Dried slices of *Dioscorea opposita* Thunb. were purchased from Bao He Tang (Jiaozuo) Pharmaceutical Co. Ltd. in Jiaozuo city, Henan provincewhereis located in the central part of China and is famous for growingDioscorea opposita for nearly 2000 years. All the chemicals and standard samples were purchased from Sigma-Aldrich Co. Ltd, USA and Tianjin Kemiou Chemical Reagent Co. Ltd, China. Analytical grade chemicals were used.

2.2. Extraction of Dioscorea opposita polysaccharides (DOP)

Four DOP samples (S1, S2, S3 and S4) were extracted and the flowchart is shown in Fig. 1. According to the extraction method of Zhang et al. (2011) with modification, the dried slices of *Dioscorea opposita* were grounded in a high speed disintegrator and sifted through a 40-mesh sieve. 1.0 kg of the dried powder was extracted twice for 3

hrs at 80 °C water bathwith 8.0 L of ethanol ($EtOH/H_2O$, 95% v/v) and then filtrated. The precipitation was extracted twice for 3 hrs at 80 °C water bath with 8.0 L of deionised water. The extracted solution was centrifuged at 4000 rpm for 10 min to remove precipitation. 1/4 volume of ethanol was added and precipitated the residue III (discarded) for 24 hrs, which was approximately equal to 20% v/v ethanol (concentration of ethanol, C_e). The supernatant I was concentrated to a 1/3 volume of primary extracted solution, and ethanol (in amount equal to four times the volume of the concentrated solution) was added and crude polysaccharide (S1) was centrifuged to obtain after 24 hrs.

The same process was operated until supernatant I, and subsequently, the crude polysaccharides were collected by grading alcohol precipitation. Firstly, the supernatant I was also concentrated to a 1/3 volume of primary extracted solution, and ethanol was added for precipitating the polysaccharides II (S2) until C_e was about to 40% v/v for 24 hrs. The polysaccharides III (S3, C_e = 60% v/v) and IV (S4, C_e = 80% v/v) were obtained by the same manner. The four samples were freeze dried for 3 days to the constant weight to determine the DOP yield and stored in vacuum desiccators over P_2O_5 for further study.

2.3. Analyses

2.3.1. Determination of glucose and protein content

Protein content was detected using Coomassie brilliant blue (Bradford, 1976) and

123 the glucose content was determined using a phenol-sulphuric acid method (Dubois et
124 al., 1956).

125 **2.3.2. Determination of molecular weight**

126 The weight-average molecular weight (Mw) and MW distributions (polydispersity
127 = Mw/Mn) of the DOP samples were measured using high performance size exclusion
128 chromatography attached to multiangle laser light scattering and refractive index
129 detector (HPSEC-MALLS-RID, Wyatt Technology Co., USA) with an OHpak SB-
130 802.5 HQ column (8.0 mm × 300 mm, Shodex Co., Japan). The mobile phase (0.1 M
131 $NaNO_3$), was pumped (Waters, 515 HPLC Pump, USA) at the flow rate of 0.5 mL/min.
132 50.0 μL of sample solution (1.8 mg/mL) was injected and the chromatogram was
133 analysed using ARTRAV software (Wyatt Technology Co., USA).

134 **2.3.3. Transmission electron microscopy (TEM)**

135 TEM (JEM-2100, JEOL Ltd., Japan) was used to inspect the size and shape of the
136 particles in the DOP sample solutions.

137 **2.4. Emulsification properties of DOP**

138 **2.4.1. Sample preparation**

139 (a) DOP samples were dissolved in deionised water (pH 7.0, conductance: 18 mΩ)
140 at different concentrations with gentle stirring at room temperature (20 °C)until

dispersed. The droplet distribution and zeta-potential were subsequently measured and compared to find the appropriate concentration (x% w/v).

(b) The dispersions of DOP (x% w/v) and GA (x% w/v) were prepared at the ratios of 1 : 1, 1 : 2, 1 : 3, 1 : 4, 2 : 3, 2 : 5 and 2 : 7.

(c) The medium-chain-triglyceride (MCT) was used as oil sample, and the ratio of GA : MCT = 1 : 1 was used according to a source which defined a “high gum-to-oil weight ratio of approximately 1:1” (Dickinson, 2003). Therefore, the ratio of DOP, GA and MCT were confirmed.

2.4.2. Emulsification measurements

The droplet diameters (z-average/polydispersity index (PDI)) and stabilities (zeta-potential) of the emulsions were investigated using Malvern zeta-potential (Malvern-NanoZS90, Malvern Ltd., UK). In order to obtain comparable and representative data, the results were recorded as the averages plus or minus the standard deviation (n = 6, ± SD).

3. Results and Discussion

3.1. Yield, glucose contents, protein contents, and MW of DOP

The yields (Y_S) of the samples, theglucose and protein content in DOP samples (%) are shown in Table 1(a). The extraction of crude polysaccharides (S1) was 4.66%, consisting of 63.25% glucose and 0.21% protein. S2, S3, and S4 were extracted by the

160 graded alcohol precipitation, and the Y_S were 2.14%, 0.48% and 1.70%, respectively.
161 Although Y_{S3} was collected the lowest, S3 obtained the highest content of glucose and
162 protein (80.13% and 0.34% respectively). In S4, there was only approximately 56.45%
163 glucose and protein. The colour of S4 was pale in ethanol, and quickly changed to
164 brown as soon as exposing to air (it was the darkest in DOP samples, shown in Fig. 1),
165 which was considered to be catecholamine and leucoanthocyanidins (Martin & Ruberté,
166 1976).

167 Table 1(a) also shows the Mw and polydispersity of DOP samples. Since the DOP
168 samples are mixed macromolecular compounds, the values of weight-average
169 molecular weight (Mw) were considered to be more reliable compared to number-
170 average molecular weight (Mn) (Rochas & Lahaye, 1989). The Mw of each DOP
171 sample was listed in descending order S1, S2, S3, and S4: 51,250 Da, 35,230 Da, 34,790
172 Da and 3,631 Da respectively. This suggested that the graded alcohol precipitation
173 separated the MW into small ranges. Mw was only one criterion. The polydispersity
174 (Mw/Mn) was another value to consider. Mw/Mn values close to 1 (1.5-2) mean the
175 distribution is narrow and the molecular weight is in a relatively small range (Xu et al.,
176 2016).

177 The detailed molecular weight distributions were shown in Table 1(b). The ranges
178 of DOP samples were 5-500 kDa, 5-200 kDa, 10-200 kDa, and 0.5-20 kDa for S1, S2,
179 S3 and S4, respectively. Each sample was distributed differently, summarised into five
180 ranges as follow:

181 (i) 0-10 kDa, 37.89% (S1), 46.82% (S2), 0% (S3) and 99.61% (S4);

(ii) 10-20 kDa, 17.62% (S1), 12.32% (S2), 48.06% (S3) and 0.39% (S4);

(iii) 20-100 kDa, 33.35% (S1), 33.32% (S2), and 46.95% (S3);

(iv) 100-200 kDa, 5.84% (S1), 5.20% (S2), 4.60% (S3).

(v) 200-500 kDa, 3.88% (S1).

As the results shown in Table 1(b), the crude polysaccharides (S1) had the widest MW range, as expected. The MW distribution of S3 was a relatively narrow range because the Mw/Mn was 1.671 (Table 1(a)) and in the range of 10-100 kDa (Table 1(b)). The distribution of S4 was 2.881 (Table 1(a)) and in the range of 0.5-20 kDa (Table 1(b)). The difference in Mw/Mn between S3 and S4 may be the results of the impurities of S4 substances containing catecholamine and leucoanthocyanidins.

3.2. Transmission Electron Microscope (TEM)

Fig. 2 shows the morphology of DOP solutions by TEM. The crude polysaccharides (S1) showed two different structures. S1-1 showed spherical particles surrounded by feather-like structures, and S1-2 showed carbohydrate branches, which proves the various structures in crude polysaccharides and explains the wide range of molecular weight. Both S2 and S3 showed globular particles, but the particles in S2 were coagulated and flocculated together while the granules in S3 were scattering and distributed. There were two different structures in Fig. 2-S4: (a) the branches of carbohydrate consisting of many small granules and (b) straight stick-like structures linked by the small granules. The micrograph of S4 showedtwo obvious distinctive structures and explainedwhy the molecular weight distributions were wider than S3.

The MW of S4 was smaller than S3, which indicates the substances in S4, such as catecholamine and leucoanthocyanidins had smaller molecular weight than polysaccharides. Results demonstrated that the extraction, purification and preparation may affect the surface topography and structure of a polysaccharide (Nep & Conway, 2010).

3.3. Emulsification properties of DOP

3.3.1. Particle sizes and zeta-potential of DOP

Table 2(a) shows the droplet diameters, PDI and zeta-potential of GA and DOP solutions in different concentrations of 0.2%, 0.4%, 0.6%, 0.8% and 1.0% w/v. Although the droplet diameters of GA were approximately from 0.16 μm to 0.28 μm, there was significant difference between 0.2%, 0.4% and 0.6%. The droplet sizes of S1 and S2 generally increased with the concentration, but the diameters of S1 droplets were decreased from 2.21μm to 1.84 μm(0.2% to 0.4% w/v, respectively). The droplet diameters of S3 from 1.57μm to 1.63 μm to 1.70μm(0.2% to 0.4% to 0.6% w/v, respectively) dropped slightly to 1.40 μm (0.8% w/v) and went up again until 1.43 μm at concentration of 1.0% w/v. The droplet diameters of S4 with high PDI were variable, and zeta-potential ranged from -16.40 mV to -20.50 mV which was also variable. The results tended to show slightly higher mean values for S1 due to the impurity. The appropriate concentration for the following study was determined to be 0.8% w/v. Overall, the droplet diameters of DOP samples showed significant differences with GA.

223 Zeta-potential is an indicator to consider the stabilities of emulsions (Williams&
224 Phillips, 2009). According to the results shown in Table 2(a), most of samples were
225 close to $\left|\pm 30\right|$. If the absolute values of zeta-potential are over 30, hydrocolloids are
226 considered to be stable; if the value of zeta-potential are less than $\left|\pm 30\right|$, hydrocolloids
227 tent to coagulated or flocculate (O'Brien et al., 1990). Therefore, GA, S1, S2 and S3
228 were considered to be stable solution with the exception of S4. The native pH values of
229 S1, S2, S3 and S4 were 6.88, 6.31, 6.71, and 6.86, respectively (data not shown). The
230 zeta-potentials for all the samples were negative which may be caused by the acidic
231 environment and by the charges of the main amino acids, aspartic acid and glutamic
232 acid.

233 **3.3.2. Droplet diameters of DOP and GA dispersions at different ratios**

234 Table 2(b) shows the droplet diameters, PDI and zeta-potential values of the
235 emulsions made of DOP and GA in different ratios (1 : 1, 2 : 3, 1 : 2, 2 : 5, 1 : 3, 2 : 7,
236 and 1 : 4, respectively). Considering both droplet diameters and zeta potential, the
237 results showed the best ratio was 1 : 1. Arabinogalactan protein complex (AGP)
238 contributes to the emulsifications of GA and consists essentially of a protein fraction
239 and about five carbohydrate "blocks"(Al-Assaf et al., 2009; Dickinson, 2003).
240 According to Zhao et al. (2005), CYP is a heteropolysaccharide with (1→3)-α-
241 glucopyranose as a main chain and -β-galactopyranose-[(1→2)-α-Mannopyranose]$_3$-
242 (1→2)-α-Mannopyranose-(1-6)- as a side chain. According to Williams & Phillips
243 (2009), the high-molecular-weight-polysaccharide-protein complex improves the

244 overall solubility with consequent benefits for emulsification properties. Thus, the
245 combination of DOP and GA may improve the emulsification properties of both. The
246 proper ratio was measured and proposed.

247 **3.3.3. Emulsification properties of DOP, GA and MCT**

248 Table 3 shows the droplet diameters (μm), PDI and zeta-potential values (mV) of
249 freshly prepared emulsions made by DOP and GA with medium chain triglycerides
250 (MCT). The distributions of peaks are shown in Fig. 3. The ratio of DOP : GA = 1 : 1
251 was chosen due to previous work (section 3.3.2), and the ratio of GA : MCT = 1 : 1 was
252 used according to research which defined a "high gum-to-oil weight ratio of
253 approximately 1:1" (Dickinson, 2003). The droplet diameter of emulsions made by GA :
254 MCT = 1 : 1 was approximately 1.78 μm, smaller than the droplet sizes of MCT (0.8%
255 w/v, 2.44 μm, data not shown). The droplet sizes of emulsions made by DOP samples
256 (S1, S2, S3 and S4) and MCT (1 : 1) were 2.17 μm, 1.22 μm, 1.55 μm and 1.38 μm,
257 respectively, which were also smaller than the size of MCT (0.8% w/v, 2.44 μm).
258 Compared to the droplet diameters of GA and MCT (1.78 μm), S2, S3 and S4 had better
259 emulsification properties with MCT. However, the zeta-potential value of S2 was -27
260 mV, which was relatively low compared to other DOP samples, but not significantly
261 different. Therefore, S2, S3 and S4 could be used as emulsifiers.

262 The glucose contents in S2 and S3 were approximately 64.43% and 80.13%
263 respectively, and the molecular weight of S2 and S3 were around 35 kDa and 34 kDa,
264 respectively (Table 1(a)). According to previous study, polysaccharide of Chinese yam

contained glucose, galactose and mannose and xylose (Zhao et al., 2005; Alves et al., 2002). Results suggested that not only protein and main chains of polysaccharides (containing glucose) contributed to the emulsifying properties, side chains (containing galactose and mannose) also contributed. Therefore, emulsions of S2 with higher molecular weight, less glucose content and protein content resulted in smaller droplet sizes. S4 (precipitation at C_e = 80%, MW ≈ 3.5 kDa) contained 56.45% glucose and protein, and other chemical substance, such as catecholamine and leucoanthocyanidins, which ultimately affected negatively on the emulsification properties of S4.

In order to investigate the emulsification properties of combinations (GA and DOP samples), the emulsification properties of DOP : GA : MCT = 1 : 1 : 1 (0.8% w/v) were studied. Table 3(III) shows the droplet diameters of emulsions made of DOP, GA and MCT (1 : 1 : 1, respectively), and only S2 showed smaller droplet sizes (0.94 μm). The droplet diameters of emulsions made of S4, GA and MCT was extremely large (18.42 μm) which may be resulted by the larger amount of small molecular weight impure chemical substances. The results showed the best emulsification properties were from S2 : GA : MCT (1 : 1 : 1), which suggests that the combination and synergistic effects of S2 and GA could improve the emulsification of both components.

4. Conclusion

Considering the tremendous focus on healthy and natural food products and the sensory evaluations required of consumers, the emulsification propertiesof polysaccharides from *Dioscorea opposita* Thunb. were studied to identify a potential

emulsifier. In addition to glucose content, protein content and MW distributions were also studied. The droplet diameters and zeta-potential of solutions made by GA and DOP in different concentrations and ratios were studied, especially the emulsions of GA and MCT (1 : 1); DOP and MCT (1 : 1); and DOP, GA and MCT (1 : 1: 1). The S2 and S3 had emulsifying properties and the emulsions made by S2 : GA : MCT (1 : 1 : 1) showed the best emulsification properties.While the beverage industry has keen interest in high quality and natural emulsifiers, DOP could be utilised as a natural emulsifier that can be improved synergistically with other emulsifiers, such as GA.

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Table 1. The yield, glucose content, protein content, molecular weight and molecular weight distributions of *Dioscorea opposita* polysaccharides (DOP)

(a) Values for the yield, glucose content, protein content, molecular weight and molecular polydispersity of DOP

	S1	**S2**	**S3**	**S4**
Yield (%)	4.66 ± 0.15	2.14 ± 0.12	0.48 ± 0.01	1.70 ± 0.10
Glucose Content (%)	63.25 ± 3.01	64.43 ± 5.18	80.13 ± 3.61	56.37 ± 6.09
Protein Content (%)	0.21 ± 0.01	0.12 ± 0.04	0.34 ± 0.01	0.08 ± 0.007
Polydispersity				
Mw/Mn	4.344	3.278	1.671	2.881
Molar mass moments (g/mol)				
Mn	11,800	10,750	20,820	1,260
Mw	51,250	35,230	34,790	3,631

Note: The results were recorded as average ± SD; Mn = number-average molecular weight; Mw = weight-average molecular weight.

(b) The molecular weight distributions of DOP

	Molecular weight Distributions (kDa)					
S1	**5－10**	**10－20**	**20－40**	**40－100**	**100－200**	**200－500**
%	37.89	17.62	20.46	12.89	5.84	3.88
S2	**5－7**	**7－10**	**10－20**	**20－40**	**40－60**	**60－100**	**100－200**
%	32.5	14.32	12.32	18.71	8.34	6.27	5.2
S3	**10－15**	**15－20**	**20－40**	**40－60**	**60－100**	**100－200**
%	26.24	21.82	28.54	9.47	8.67	4.6
S4	**0.5－1**	**1－2**	**2－5**	**5－10**	**10－20**
%	40.4	20.96	21.21	17.04	0.39

Table 2. Droplet diameters (z-average, µm), polydispersity index (PDI), and zeta-potential (mV) of the solutions made of GA/MCT/DOP samples at different concentrations (a), and different ratios of DOP with GA (b)

Droplet diameters (z-average µm ± standard deviation and mean PDI in parentheses)					
	Concentrations (% w/v)				
	0.2%	**0.4%**	**0.6%**	**0.8%**	**1.0%**
GA	0.16 ± 0.02^{a} (0.43)	0.28 ± 0.04^{ab} (0.53)	0.20 ± 0.01^{bc} (0.54)	0.28 ± 0.03^{acd} (0.57)	0.29 ± 0.01^{ace} (0.38)
S1	2.21 ± 0.06^{af} (0.53)	1.84 ± 0.08^{bfg} (0.49)	1.85 ± 0.08^{cfh} (0.93)	1.86 ± 0.02^{dfi} (0.28)	1.87 ± 0.04^{efj} (0.21)
S2	0.61 ± 0.07^{afk} (0.39)	0.63 ± 0.06^{bgl} (0.52)	0.67 ± 0.07^{chklm} (0.39)	0.73 ± 0.02^{diklmn} (0.30)	0.74 ± 0.07^{ejklmo} (0.28)
S3	1.57 ± 0.06^{afkp} (0.41)	1.63 ± 0.03^{bglq} (0.18)	1.70 ± 0.10^{cmr} (0.28)	1.40 ± 0.06^{dinpqrs} (0.23)	1.43 ± 0.06^{ejopqrt} (0.16)
S4	0.76 ± 0.10^{afkpu} (0.77)	0.67 ± 0.04^{bgqv} (0.75)	1.52 ± 0.01^{chmuvw} (0.51)	0.87 ± 0.06^{dinsvwx} (0.68)	1.32 ± 0.01^{ejotuvwx} (0.35)
Zeta-potential (mV)					
	Concentrations (% w/v)				
	0.2%	**0.4%**	**0.6%**	**0.8%**	**1.0%**
GA	-27.70 ± 3.27	-28.70 ± 0.66	-24.47 ± 0.56	-21.90 ± 0.53	-22.80 ± 0.53
S1	-27.60 ± 0.46	-24.88 ± 0.43	-23.97 ± 0.57	-22.77 ± 0.86	-21.63 ± 0.25
S2	-30.30 ± 0.95	-26.70 ± 1.25	-26.33 ± 0.57	-24.87 ± 0.83	-23.60 ± 0.54
S3	-29.95 ± 0.82	-27.83 ± 0.75	-27.28 ± 0.67	-25.40 ± 0.22	-23.92 ± 0.65
S4	-18.43 ± 1.21	-20.50 ± 0.26	-22.20 ± 1.05	-16.57 ± 0.55	-16.40 ± 1.18

(a) Droplet diameters (μm), PDI and zeta-potential (mV) of GA and DOP solutions at different concentrations

Note: Data are reported as the mean of 6 replicates, and the results are presented as the mean ± SD. Paired symbols a to x showed significant difference ($P < 0.05$)

(b) Droplet diameters (z-average, μm), PDI and zeta-potential (mV) of the emulsion made by DOP and GA at different ratios (Concentrations of DOP = 0.80% w/v)

Droplet diameters (z-average μm ± standard deviation and mean PDI in parentheses)

	Ratios of DOP : GA						
	1 : 1	**2 : 3**	**1 : 2**	**2 : 5**	**1 : 3**	**2 : 7**	**1 : 4**
S1	1.65 ± 0.04 (0.30)	2.12 ± 0.07 (0.59)	2.06 ± 0.07 (0.35)	3.20 ± 0.03 (0.58)	4.33 ± 0.07 (0.54)	5.30 ± 0.07 (0.37)	4.34 ± 0.07 (0.45)
S2	1.52 ± 0.04 (0.47)	2.30 ± 0.12 (0.57)	3.92 ± 0.09 (0.54)	1.71 ± 0.06 (0.50)	2.32 ± 0.06 (0.57)	1.22 ± 0.09 (0.40)	1.85 ± 0.03 (0.44)
S3	1.87 ± 0.07 (0.44)	2.15 ± 0.05 (0.17)	1.89 ± 0.06 (0.30)	5.08 ± 0.09 (0.38)	6.12 ± 0.03 (0.46)	4.51 ± 0.10 (0.73)	4.46 ± 0.05 (0.19)
S4	0.29 ± 0.10 (0.73)	0.33 ± 0.09 (0.67)	0.56 ± 0.03 (0.94)	0.29 ± 0.00 (0.57)	0.26 ± 0.04 (0.69)	0.26 ± 0.02 (0.66)	0.55 ± 0.02 (0.87)

Zeta-potential (mV)

	Ratios of DOP : GA						
	1 : 1	**2 : 3**	**1 : 2**	**2 : 5**	**1 : 3**	**2 : 7**	**1 : 4**
S1	-29.33 ± 0.40	-29.01 ± 0.55	-27.30 ± 0.17	-26.67 ± 0.38	-25.07 ± 0.29	-23.8 ± 0.95	-22.70 ± 0.30
S2	-29.40 ± 0.30	-29.01 ± 0.58	-28.30 ± 0.20	-28.13 ± 0.55	-28.13 ± 0.31	-29.83 ± 0.95	-28.30 ± 0.26
S3	-24.23 ± 0.42	-28.47 ± 0.42	-23.73 ± 0.45	-27.60 ± 0.66	-27.60 ± 0.66	-29.67 ± 0.70	-22.50 ± 0.72
S4	-23.63 ± 0.50	-21.17 ± 0.55	-21.30 ± 0.17	-21.00 ± 0.85	-20.37 ± 0.85	-20.17 ± 0.35	-19.90 ± 0.52

Note: Data are reported as the mean of 6 replications, and the results are presented as the mean ± SD

Table 3. Droplet diameters (z-average, μm), polydispersity index (PDI), and zeta-potential (mV) of emulsions made of GA and MCT, DOP and MCT, and DOP, GA and MCT

	(I) GA		(II) MCT		(III) GA + MCT	
	z-average (d. μm) (mean PDI)	**zeta-potential (mV)**	**z-average (d. μm) (mean PDI)**	**zeta-potential (mV)**	**z-average (d. μm) (mean PDI)**	**zeta-potential (mV)**
MCT	1.78 ± 0.09^{a} (0.16)	-29.08 ± 0.97				
S1	1.65 ± 0.04^{b} (0.30)	-29.33 ± 0.40	2.17 ± 0.08^{abf} (0.56)	-27.00 ± 0.40	2.33 ± 0.06^{abj} (0.40)	-31.47 ± 0.81
S2	1.52 ± 0.04^{abc} (0.47)	-29.40 ± 0.30	1.22 ± 0.06^{acfg} (0.57)	-29.90 ± 0.75	0.94 ± 0.05^{acgjk} (0.49)	-29.47 ± 1.27
S3	1.87 ± 0.07^{bcd} (0.44)	-24.23 ± 0.42	1.55 ± 0.06^{adfgh} (0.28)	-29.20 ± 0.36	2.48 ± 0.05^{adhkl} (0.53)	-29.30 ± 0.30
S4	0.29 ± 0.10^{abcde} (0.73)	-23.63 ± 0.50	1.38 ± 0.02^{aefghi} (0.84)	-29.00 ± 0.96	18.42 ± 0.44^{aeijkl} (0.34)	-27.53 ± 0.12

Note: The concentration of each sample was 0.8% w/v. The samples consisted of : (i) GA and MCT; (ii) GA : MCT = 1 : 1; (iii) DOP : GA = 1 : 1; (iv) DOP : MCT = 1 : 1; and (v) DOP : GA: MCT = 1 : 1 : 1. Data are reported as the mean of 6 replicates, and the results are presented as the mean ± standard deviation. Paired symbols a to l showed significant difference ($P < 0.05$)

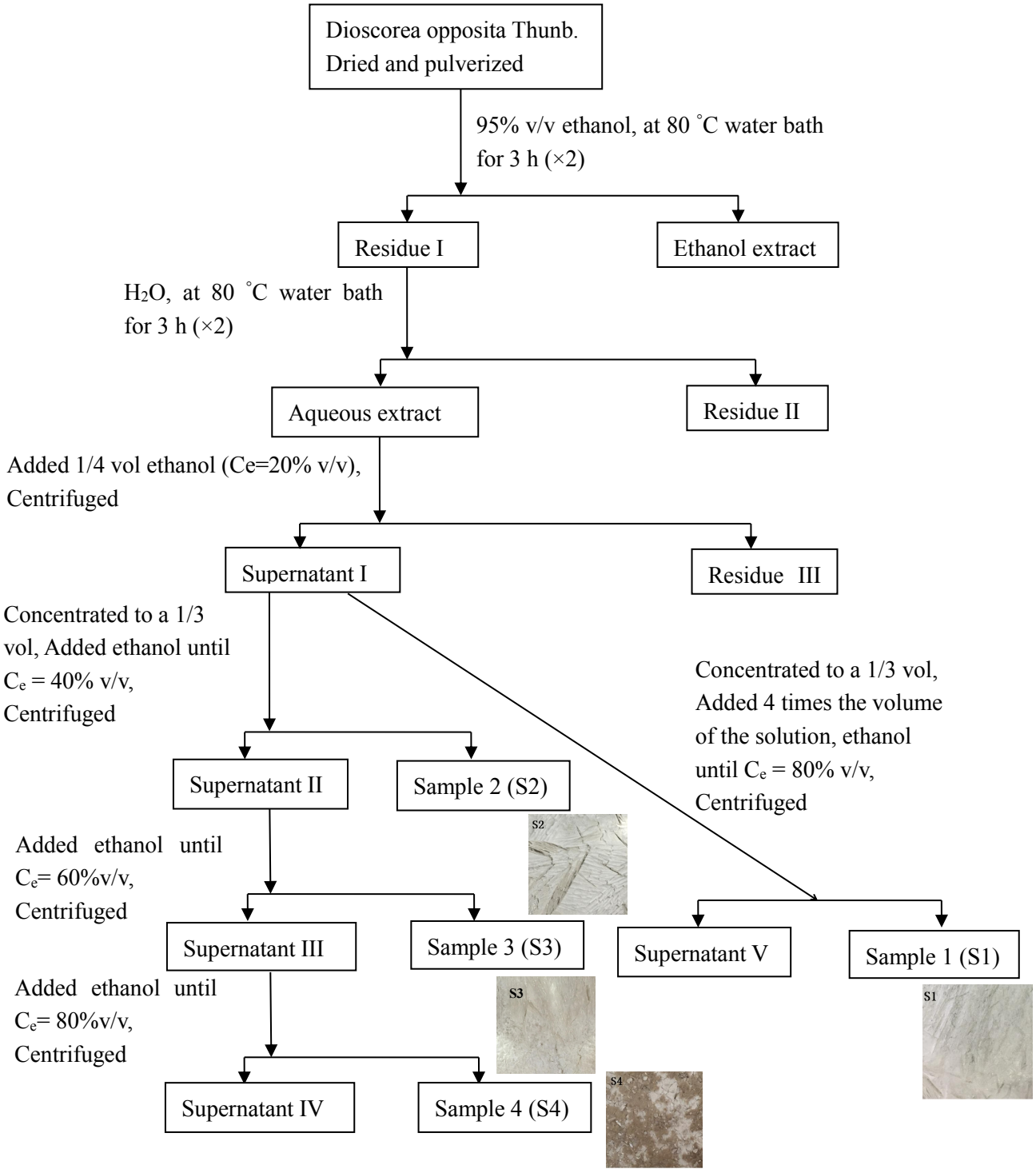

Fig. 1. Flowchart describing the extraction of *Dioscorea opposita* polysaccharides

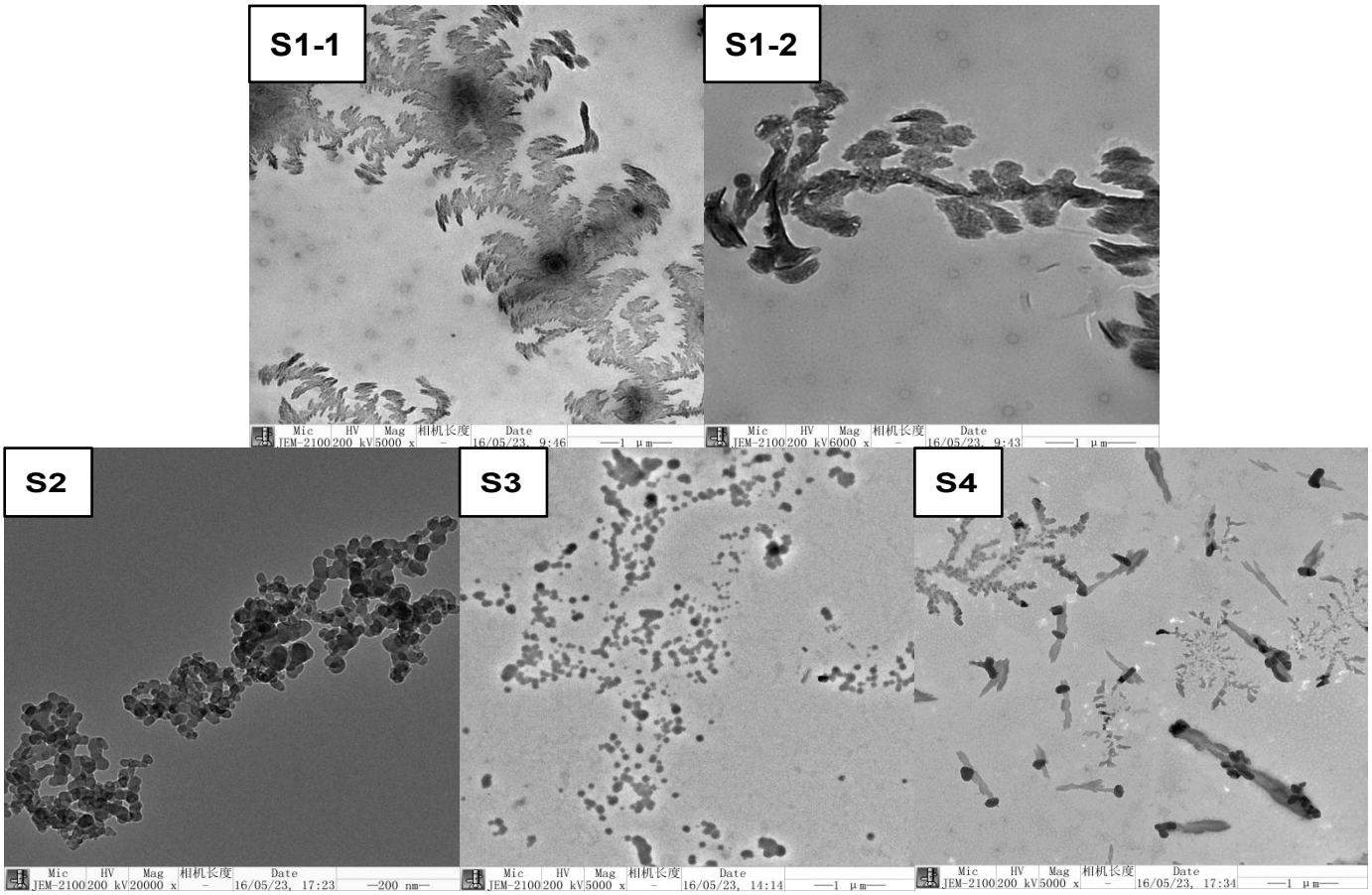

Fig. 2. Micrographs of *Dioscorea opposita* polysaccharide solutions by TEM. S1, S3 and S4 are shown at a magnification of ×5000 and S2 is shown at a magnification of ×20,000

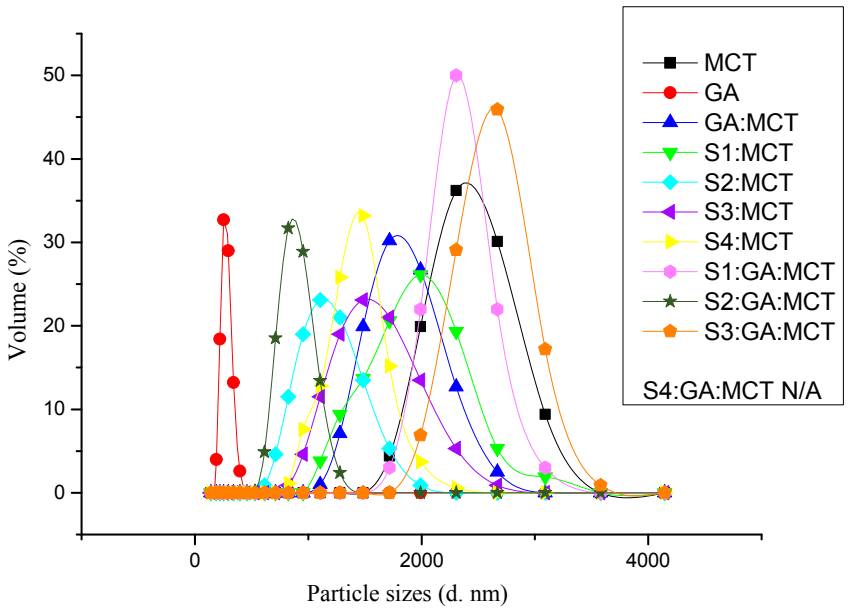

Fig. 3. Droplet sizes and distributions of the freshly prepared emulsions. The concentration of each sample was 0.8% w/v, including MCT, GA, GA : MCT = 1 : 1; DOP : MCT = 1 : 1, and DOP : GA : MCT = 1 : 1 : 1. The droplet diameter of the emulsion made by S4 : GA : MCT = 1 : 1 : 1 was too large (not shown).

N/A = not available; Data was used as mean from 6 replications