Chemical components and emulsification properties of mucilage from *Dioscorea opposita* Thunb.

Running Title: Characteristics and Emulsifications of Chinese yam mucilage

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Abstract

The properties of mucilage obtained from *Dioscorea opposita*, generated during industrial manufacturing were investigated in this study. Characteristics such as monosaccharide content, amino acid content, molecular weight, and structural features were measured, whereas morphology was observed using a scanning/transmission electron microscope. Additionally, emulsification properties at different concentrations (0.2%, 0.4%, 0.6%, 0.8%, and 1.0%) and under acidic and basic pH (5.0 and 9.0) conditions were studied. The results showed that emulsions prepared from mucilage and medium-chain triglycerides presented more effective emulsifying functions and higher stability, especially at low concentrations. Both, acidic and basic conditions improved the overall emulsification properties, which suggested that the isoelectric point of amino acids may be involved in the emulsification properties. The results of this study show that mucilage from *Dioscorea opposita* can be considered as a sustainable resource of a natural emulsifier obtained from industrial waste.

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

1. Introduction

The yam (Family *Dioscoreaceae*) is an important tropical root used as a functional food as well as a source for natural medicine due to several pharmacological activities (Huang et al., 2011). *Dioscorea opposita* Thunb. is a kind of Chinese yams (CY) that is rich in starch, water-soluble polysaccharides, and mucilage (Herlina, 2015). Mucilage defined as a polysaccharide with unique viscosity characteristics is widely used in the pharmaceutical and food industries as a thickening agent and emulsion stabiliser (Lee et al., 2003). According to Kilho et al. (1985) and Ohtani & Murakami (1991), the water-soluble mucilage from *Dioscorea batatas* Dence is rich in glucomannan. Myoda et al. (2006) studied the interaction between mannan and soluble proteins in *Dioscorea opposita* mucilage (DOM), which affects the viscosity of DOM. Several pharmacological effects of Chinese yam mucilage (CYM) have been reported, including antioxidant, enzyme inhibitory, and antimutagenic activities (Lee et al., 2003; Hsu, et al., 2003; Zhang et al., 2016).

Emulsifying agents consist of a water-soluble polar component (hydrophilic) and a non-polar, water-insoluble component (hydrophobic).These agents are important in the food industry as they improve the sensory quality, flavour, texture, palatability, mouthfeel, and general appearance of the final products (Dickinson & Stainsby, 1988). Previous studies have reported that mucilage from various plants such as yellow mustard and chia (*Salvia hispanica* L.) have emulsification and/or stabilisation properties (Wu et al., 2015; Capitani et al., 2016). Therefore, in this study we investigated the emulsification properties of DOM which is a potential candidate for

food emulsifier.

Usually harvested in November, *Dioscorea opposita* is a seasonal crop with a short shelf-life, as it contains protein and steroidal saponins, which reduce the quality of the yam during storage (Yang & Lin, 2008; Xue et al., 2015). Therefore, dried slices of *Dioscorea opposita* are prepared on an industrial scale. However, DOM generated during industrial processing is discarded (Li et al., 2016). DOM is a high-yielding, natural product that is easily extracted and used as an additive in food applications and functional food products. Medium-chain triglyceride (MCT) is used as a fat/lipid carrier in food flavours, essences, and pigments, which are widely used in the food industry (Télessy et al., 2009). Hence, in this study, the oil/water (O/W) emulsion was made by emulsification using MCT.

Gum arabic (GA), one of the most extensively used exudate gums, is a naturally-occurring complex polysaccharide with small amount of protein (2%-3%), which displays both emulsifying and emulsion stabilising properties (McClements, 2005; Ma et al., 2015). Therefore, the aim of this study was to determine the chemical composition and examine the emulsification properties of DOM in an oil-in-water emulsion with GA, in order to identify the main chemical components that contribute to the emulsifying property.

2. Materials and methods

2.1. Materials

Fresh *Dioscorea opposita* Thunb. was purchased in November 2015 from Bao He Tang (Jiaozuo) Pharmaceutical Co. Ltd., Jiaozuo city, Henan province, a farm located in

87 Central China and known for *Dioscorea opposita* cultivation since approximately 2000
88 years. All reagents and standard samples including GA (*Acacia senegal*, G-9752) were
89 purchased from Sigma-Aldrich Co. Ltd, USA, and Tianjin Kemiou Chemical Reagent
90 Co. Ltd, China. All chemicals used were of analytical grade.

91 **2.2. Extraction of *Dioscorea opposita* mucilage (DOM)**

92 DOM was extracted as previously described by Andrade et al. (2015) with minor
93 modifications. Briefly, approximately 4.0 kg fresh *Dioscorea opposita* was washed,
94 peeled, and washed again in deionised water (pH 7.0, conductance: 18 mΩ).
95 Approximately 300 g portions of *Dioscorea opposita* were sliced and ground in an
96 industrial blender for 5 min. All portions were subsequently pooled and homogenised.
97 After centrifugation at 4,000 rpm for 5 min. DOM was collected in the supernatant and
98 freeze-dried for 3 days to a constant weight to determine DOM yield. DOM was stored
99 in vacuum desiccators over P_2O_5 until use.

100 **2.3. Analytical methods**

101 **2.3.1. Determination of glucose and protein content**

102 Glucose content and protein content were determined using phenol-sulphuric acid
103 method and Coomassie brilliant blue method, respectively (Dubois et al., 1956;
104 Bradford, 1976).

105 **2.3.2. Determination of monosaccharides**

106 As previously described by Andrade et al., (2015), gas chromatography-mass
107 spectrometry (GC-MS, ThermoFisher Trace 1310 ISQ) was used for the quantitative
108 determination of monosaccharides with HP-5MS (30 m × 0.25 mm × 0.25 μm). A total

of 8 standards (Ludger Co. Ltd) including fucose, arabinose, rhamnose, galactose, glucose, mannose, xylose, and fructose were used to determine the monosaccharides in DOM.

2.3.3. Determination of amino acids

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

2.3.4. Determination of molecular weight (MW)

The weight-average MW (Mw) and MW polydispersity (Mw/Mn) of DOM samples were measured using high-performance size-exclusion chromatography attached to multiangle laser light scattering and refractive index detector (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 (0.1 M $NaNO_3$) was pumped (Waters, 515 HPLC Pump, USA) at a flow rate of 0.5 mL/min, 50.0 μL of sample solutions (1.8 mg/mL) was injected, and the chromatogram was analysed by using ARTRAV software (Wyatt Technology Co., USA).

2.3.5. pH determination

DOM (1% w/v) was prepared and the pH meter (ZD-2A, Dapu Instrument, Shanghai, China) was calibrated using standard solutions of known pH (4.00, 6.86 and 9.18). The pH value of the sample solutions was read directly from the instrument and the mean value of two consecutive measurements was recorded.

2.3.6. Fourier transform infrared spectroscopy (FT-IR)

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

2.3.7. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

A thermal field emission scanning electron microscope (JSM-7001F, JEOL Ltd., Japan) was used to inspect the morphology of DOM, and transmission electron microscope (JEM-2100, JEOL Ltd., Japan) was used to inspect the size and shape of the particles in the DOM solution.

2.4. Emulsification properties of DOM

2.4.1. Sample preparation

Each sample of DOM, GA, and MCT was separately dissolved in deionised water (pH 7.0, resistivity: 18 mΩ) at different concentrations (0.2%, 0.4%, 0.6%, 0.8% and 1.0% w/v) with gentle stirring at room temperature (20 °C) until dispersion.

As previously described by Ma et al. (2015), DOM was dispersed (10% w/v) by adding the required amount of sample to deionised water with gentle stirring at room temperature (20 °C). The solutions were further degassed under vacuum to remove any entrapped air bubbles. DOM samples were prepared by either dialysing overnight at 4 °C (native) or dialysing against phosphate-buffered solutions of various pH (0.3 M, pH 5.0, pH 7.0, and pH 9.0) overnight at 4 °C to equilibrate to the required pH. Part of the samples was freeze-dried and stored in vacuum desiccators over P_2O_5 for further

152 study. The remaining samples were then dialysed against several changes of deionised
153 water for 24 hrs at 4 °C. No change in sample volume was observed. Materials were
154 freeze-dried and stored in vacuum desiccators over P_2O_5 for further study.

155 **2.4.2. Droplet distribution measurements**

156 The droplet diameters (z-average) and distribution (polydispersity index, PDI)
157 and zeta-potential of emulsions were measured using Malvern zeta-potential (Malvern-
158 NanoZS90, Malvern Ltd., UK). In order to obtain comparable and representative data,
159 the results were recorded as the averages of 6 replicates ± standard deviation (SD).

160 # 3. Results and Discussion

161 ## 3.1. Components of DOM

162 **Table 1.** Characterisation, monosaccharides, amino acid content, and molecular weight
163 of *Dioscorea opposita* mucilage

164 (a) Characterisation and monosaccharides of *Dioscorea opposita* mucilage

Characteristics	**Average ± SD**
Yield (%)	8.18 ± 0.08
Moisture (%)	64.59 ± 0.07
Glucose Content (%)	16.00 ± 0.06
Protein Content (%)	2.78 ± 0.48
Ash (%)	16.00± 0.12
pH	6.96 ± 0.02
Monosaccharides (%)	
Rhamnose	0.25
Arabinose	0.54
Xylose	5.38
Mannose	33.40
Glucose	49.50
Galactose	10.90
Uronic acid	ND

165 **Note:** ND = None detected; SD = standard deviation; fucose, galacturonic acid, and

166 glucuronic acid were tested and found below analytical detection limit.

167

168 (b) Amino acid composition, mean retention time (RTm) and peak area of *Dioscorea*

169 *opposita* mucilage

Amino Acid	Content (%)	RTm (min)	Peak Area ($\times 10^7$)
Aspartic acid (ASP)	4.16	5.18	5.73
Threonine (THR)	1.57	5.70	2.65
Serine (SER)	3.08	6.23	7.03
Glutamic acid (GLU)	4.55	7.01	7.10
Glycine (GLY)	1.38	10.11	3.61
Alanine (ALA)	1.73	10.91	4.45
Cysteine (CYS)	0.19	12.03	0.18
Valine (VAL)	1.69	12.63	3.23
Methionine (MET)	0.56	13.97	0.83
Isoleucine (ILE)	1.37	16.25	2.05
Leucine (LEU)	2.53	17.40	3.91
Tyrosine (TYR)	0.90	18.56	1.05
Phenylalanine (PHE)	1.96	19.47	2.47
Lysine (LYS)	1.71	21.57	2.70
Tryptophan (TRP)	0.56	22.68	0.83
Histidine (HIS)	0.81	23.75	1.10
Arginine (ARG)	4.35	28.44	4.29
Proline (PRO)	0.82	30.73	0.25

170

171 (c) The molecular weight and distribution of *Dioscorea opposita* mucilage

MW factors of *Dioscorea opposita* mucilage					
Polydispersity		**Molar mass moments (g/mol)**			
Mw/Mn	**Mz/Mn**	**Mn**	**Mp**	**Mw**	**Mz**
6.715	238.841	21,390	12,610	143,700	511,000
MW distributions (kDa)					
10-15	**15-20**	**20-40**	**40-100**	**100-200**	**200-500**
35.48%	17.06%	16.92%	10.37%	5.99%	8.12%

172 **Note:** Mn = number-average MW; Mp = peak-average MW; Mw = weight-average

MW; Mz = z-average MW.

Table 1(a) shows the characterisation including yield, moisture, glucose content, protein content, ash, pH value, and monosaccharide composition of DOM. The yield of DOM was 8.18%, including 64.59% moisture, 16.00% glucose, 2.78% protein, and 16.00% ash. Previous studies reported an yield of 9.63% and 4.20% for taro and bird's nest fern (*Asplenium australasicum*) mucilage, respectively (Andrade et al., 2015; Zeng & Lai, 2016). Therefore, DOM yield in this study was of a reasonable value. The monosaccharides found in DOM were as follows in descending order: glucose, mannose, galactose, xylose, arabinose, and rhamnose (49.50% > 33.40% > 10.90% > 5.38% > 0.54% > 0.25%, respectively), while uronic acid was not detected. Three monosaccharides, glucose, mannose and galactose constituted approximately 93.8% of polysaccharide content, which could be in the form of a high concentration of glucomannan and galactomannan. On the other hand, GA, a commercial emulsifier containing > 97% polysaccharide and 2.5% protein, was used as a competitive control sample. GA is a member of the arabinogalactan-protein group and is a complex, branched heteropolyelectrolyte, with a backbone of 1,3-linked β-galactopyranose units and side-chains of 1,6-linked galactopyranose units terminating in a glucuronic acid or a 4-O-methylglucuronic acid residue (Dickinson, 2003).

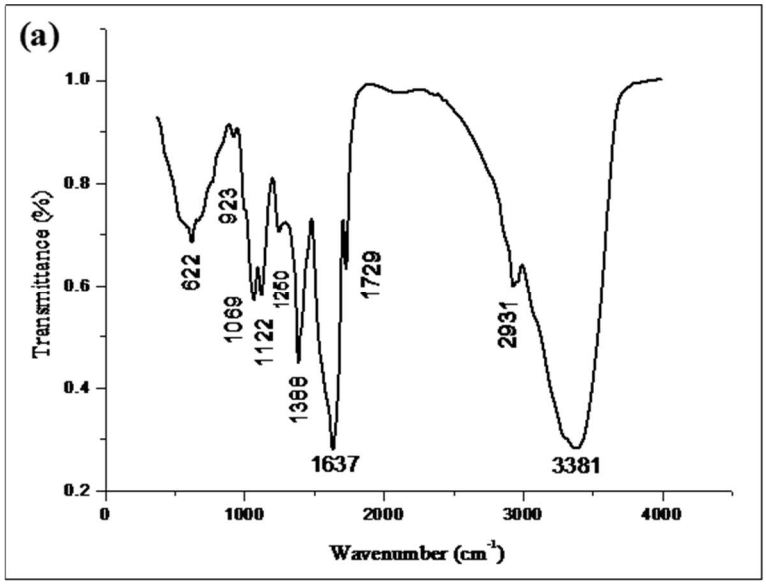
Table 1(b) shows the amino acid content, mean retention time (RTm) and peak area of each amino acid found in DOM. A total of 18 types of amino acids were detected, including acidic polar amino acids with negative charge [such as glutamic acid (4.55%)

and aspartic acid (4.16%)], basic polar amino acids with positive charge [such as arginine (4.35%) and lysine (1.71%)], and neutral charge amino acid [such as serine (3.08%), leucine (2.53%), phenylalanine (1.96%), alanine (1.73%), valine (1.69%), threonine (1.57%), glycine (1.38%), and isoleucine (1.37%)] (Damodaran et al., 1996). Glutamate is commonly found in food and is known for its beneficial functions, such as improving food flavour, enhancing food intake, and excitatory neurotransmitter activity (Jinap & Hajeb, 2010; Bellisle, 1999). In the 1970s, aspartic acid racemisation was used to measure human dentine and monitor lens cataract formation during aging (Helfman & Bada, 1976; Masters et al., 1977). Similarly, *Dioscorea opposita* anorexic and antioxidant effects, possibly contributed by glutamate and aspartic acid. Previous studies have also suggested that arginine may contribute to seminal emission functions (Food Chemistry, submitted).

Detailed molecular weight polydispersity and distribution are shown in Table 1(c). Since DOM is a macromolecular compound, MW was determined in terms of Mw (143,700 Da), which was relatively more reliable than number-average molecular weight (Mn). The PDI (Mw/Mn) was 6.715, indicating a broad range of molecular weight distribution (10-500 kDa). The results show that DOM contains 52.54% macromolecules of size < 20 kDa, 27.29% macromolecules of size between 20 and 100 kDa, and 14.11% macromolecules of size > 100 kDa. A previous study showed that crude polysaccharides in *Dioscorea opposita* comprised of approximately 55.51% macromolecules of size 0-20 kDa (Food Chemistry, Submitted). These results suggest

that although MW of DOM much higher than that of *Dioscorea opposita* crude polysaccharides, DOM contains a smaller proportion of smaller macromolecules.

3.2. Characteristics of *Dioscorea opposita* mucilage



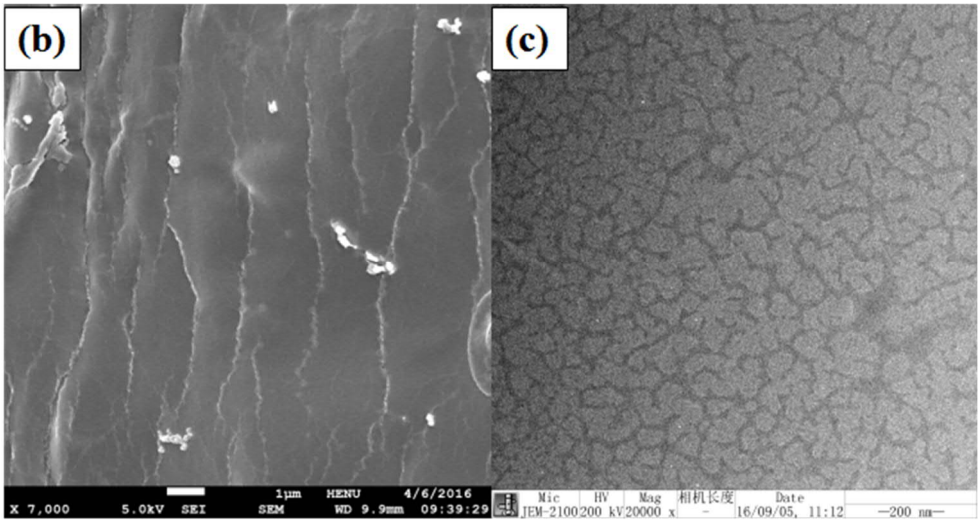


Fig. 1. Characterisation of *Dioscorea opposita* mucilage (DOM)

(a) Fourier transform infrared spectra of DOM; (b) Scanning electron microscopic image of DOM at magnifications of ×7000; (c) transmission electron microscopic image of DOM at magnifications of ×20,000

3.2.1. FTIR

Fig. 1(a) shows the FTIR for DOM. The wide band at 3381 cm^{-1} indicates hydroxyl groups, and that at 2931 cm^{-1} indicates CH bond. The peak at 1729 cm^{-1} corresponds to carbonyl (C=O) in carboxylic acids, aldehydes, and ketones (Andrade et al., 2015). The wave number at 1637 cm^{-1} indicates the functional group of amide I band, mainly due to the C=O stretching of peptide groups. The peaks at 1388 cm^{-1} and 1250 cm^{-1} indicate methyl group (CH_3) and C-O stretching of carboxylic acids, respectively. Compared with FTIR of polysaccharides from *Dioscorea opposita*, no peak was observed for C-O-H of carboxylic acid (noted in the range of 1395-1440 cm^{-1}) for DOM (Food Chemistry, submitted).

3.2.2. SEM & TEM

Surface morphology images for DOM in the powder form, analysed by SEM and in solution, analysed by TEM are shown in Fig. 1(b) and (c), respectively. Previous studies show that surface topography, structure, and properties of polysaccharides may be influenced by the conditions of extraction, purification, and preparation (Nep & Conway, 2010). DOM powder showed squamous structure, while DOM solution resembled a cracked film, similar to parched earth. DOM solution is viscous, thick, and easily forms a film. However, the concentration of mucilage in this study was low, which caused a relative decrease in cohesiveness, resulting in the cracked morphology, as shown in Fig. 1(c).

Table 2. Droplet diameter (μm) and zeta-potential (mV) of solution of gum arabic (GA) and *Dioscorea opposita* mucilage (DOM) at different concentrations

(a) Droplet diameter (μm) and polydispersity index (PDI) of GA and DOM solutions at different concentrations

Droplet diameter (z-average in μm ± standard deviation with 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^{abc} (0.54)	0.28 ± 0.03^{acd} (0.57)	0.29 ± 0.01^{ace} (0.38)
DOM-N	0.86 ± 0.06^{af} (0.56)	0.93 ± 0.08^{bg}(0.57)	1.09 ± 0.09^{cfgh} (0.54)	1.25 ± 0.06^{dfghi} (0.39)	1.45 ± 0.04^{efghij} (0.46)
DOM-pH 7	1.56 ± 0.09^{afk} (0.45)	2.48 ± 0.10^{bgkl} (0.47)	2.85 ± 0.07^{chklm} (0.51)	3.23 ± 0.06^{diklmn} (0.39)	5.56 ± 0.11^{ejklmno} (0.46)
DOM-pH 5	1.34 ± 0.02^{afkp} (0.39)	1.43 ± 0.09^{bglq} (0.62)	1.44 ± 0.02^{chmr} (0.51)	1.56 ± 0.04^{dinps} (0.53)	1.59 ± 0.12^{eopt} (0.36)
DOM-pH 9	0.58 ± 0.02^{afkpu} (0.57)	0.68 ± 0.01^{bglquv} (0.59)	0.85 ± 0.03^{chmruvw} (0.56)	1.02 ± 0.03^{dinsuvwx} (0.54)	$1.24 \pm 0.04^{ejotuvwxy}$ (0.53)
DOM-pH 5-7	2.46 ± 0.10^{afkpu} (0.49)	3.12 ± 0.08^{bglqv} (0.36)	3.18 ± 0.07^{chmrw} (0.25)	4.24 ± 0.08^{dinsx} (0.30)	4.85 ± 0.37^{ejoty} (0.32)
DOM-pH 9-7	1.06 ± 0.09^{afkpu} (0.22)	1.44 ± 0.01^{bglv} (0.46)	1.53 ± 0.05^{chmw} (0.50)	2.30 ± 0.09^{dinsx} (0.34)	2.79 ± 0.08^{ejoty} (0.44)

Note: DOM-N = native DOM; Data are reported as mean of 6 replicates; Results are presented as mean ± standard deviation; Paired values with superscript letters a through y indicate significant difference ($P < 0.05$).

(b) Zeta-potential (mV) of GA and DOM solutions at different concentrations

	Concentrations (%w/v)				
	0.2%	**0.4%**	**0.6%**	**0.8%**	**1.0%**
GA	-27.70 ± 3.27	-28.70 ± 0.66	-24.47 ± 2.56	-21.90 ± 0.53	-22.80 ± 0.53
DOM-N	-45.90 ± 1.68	-44.68 ± 0.87	-45.57 ± 1.07	-46.67 ± 1.61	-51.48 ± 0.81
DOM-pH 7	-47.50 ± 1.51	-47.33 ± 1.36	-49.60 ± 1.51	-53.50 ± 1.31	-57.00 ± 1.65
DOM-pH 5	-47.37 ± 3.29	-40.47 ± 0.59	-40.60 ± 0.26	-38.73 ± 1.29	-37.97 ± 1.67
DOM-pH 9	-38.83 ± 1.27	-39.43 ± 1.80	-38.77 ± 0.32	-40.80 ± 0.98	-44.10 ± 0.30
DOM-pH 5-7	-55.80 ± 2.60	-56.97 ± 2.23	-56.23 ± 0.86	-55.57 ± 1.00	-54.87 ± 2.50
DOM-pH 9-7	-45.87 ± 3.25	-54.47 ± 2.23	-64.00 ± 3.22	-70.80 ± 2.78	-60.80 ± 5.97

Note: DOM-N = native DOM; Data are reported as mean of 6 replicates; Results are presented as mean ± standard deviation.

253 **Table 3.** Droplet diameter (μm) and zeta-potential (mV) of emulsions made from *Dioscorea opposita* mucilage (DOM) and medium-chain

254 triglycerides (MCT) at different concentrations

255 (a) Droplet diameter (μm) and polydispersity index (PDI) of emulsions made from DOM and MCT at different concentrations

Droplet diameters (z-average in μm± standard deviation with mean PDI in parentheses)					
	Concentrations (% w/v)				
	0.2%	**0.4%**	**0.6%**	**0.8%**	**1.0%**
MCT	2.89 ± 0.07^{a} (0.35)	1.94 ± 0.03^{ab} (0.45)	2.19 ± 0.01^{abc} (0.54)	2.44 ± 0.04^{abcd} (0.89)	2.68 ± 0.01^{abcde} (0.61)
GA + MCT	1.38 ± 0.05^{af} (0.30)	1.21 ± 0.07^{bfg} (0.16)	1.28 ± 0.02^{cfh} (0.32)	1.78 ± 0.09^{dfghi} (0.16)	1.68 ± 0.06^{efghj} (0.06)
DOM -N + MCT	1.04 ± 0.07^{afk} (0.39)	1.15 ± 0.02^{bl} (0.17)	1.74 ± 0.03^{chklm} (0.15)	1.74 ± 0.01^{dkln} (0.19)	2.52 ± 0.32^{jklmno} (0.19)
DOM-pH 7 + MCT	1.16 ± 0.06^{afp} (0.54)	1.38 ± 0.05^{bglpq} (0.34)	1.95 ± 0.05^{chmpqr} (0.20)	2.15 ± 0.12^{dinpqs} (0.43)	2.38 ± 0.09^{ejpqrt} (0.32)
DOM-pH 5 + MCT	1.16 ± 0.09^{af} (0.47)	1.04 ± 0.10^{bgq} (0.34)	1.05 ± 0.04^{chmr} (0.17)	0.94 ± 0.05^{dins} (0.20)	1.07 ± 0.03^{ejot} (0.30)
DOM-pH 9 + MCT	0.39 ± 0.01^{afkp} (0.23)	0.41 ± 0.01^{bglq} (0.20)	0.43 ± 0.02^{chmr} (0.16)	0.47 ± 0.02^{dins} (0.14)	0.54 ± 0.04^{ejot} (0.25)
DOM-pH 5-7 + MCT	1.62 ± 0.08^{afkp} (0.44)	$2.21 \pm 0.06b^{glq}$ (0.16)	2.28 ± 0.08^{hmr} (0.22)	3.56 ± 0.06^{dins} (0.35)	3.80 ± 0.02^{ejot} (0.28)
DOM-pH 9-7 + MCT	0.94 ± 0.06^{afp} (0.28)	1.80 ± 0.09^{glq} (0.64)	2.38 ± 0.06^{chmr} (0.55)	2.96 ± 0.06^{dins} (0.36)	3.72 ± 0.09^{ejot} (0.49)

256 **Note:** DOM-N = native DOM; Data are reported as mean of 6 replicates; Results are presented as mean ± standard deviation; Paired values with

257 superscript letters a through t indicate significant difference ($P < 0.05$).

(b) Zeta-potential (mV) of emulsions made from DOM and MCT at different concentrations

	Concentrations (%w/v)				
	0.2%	**0.4%**	**0.6%**	**0.8%**	**1.0%**
MCT	-32.38 ± 0.45	-32.83 ± 2.50	-35.20 ± 0.62	-35.30 ± 0.80	-30.80 ± 1.14
GA + MCT	-38.17 ± 2.65	-34.80 ± 0.87	-29.70 ± 0.10	-29.01 ± 0.97	-27.75 ± 1.42
DOM-N + MCT	-49.88 ± 0.70	-44.38 ± 1.33	-44.77 ± 0.06	-41.97 ± 1.16	-45.17 ± 0.91
DOM-pH 7 + MCT	-47.83 ± 1.82	-42.60 ± 1.65	-43.40 ± 1.35	-46.70 ± 0.95	-46.47 ± 1.04
DOM-pH 5 + MCT	-46.00 ± 0.72	-41.80 ± 1.47	-41.97 ± 0.67	-40.60 ± 0.87	-40.83 ± 0.25
DOM-pH 9 + MCT	-57.10 ± 1.59	-51.43 ± 2.07	-46.57 ± 1.11	-43.30 ± 0.35	-40.83 ± 1.46
DOM-pH 5-7 + MCT	-55.30 ± 3.88	-52.87 ± 1.50	-56.90 ± 1.15	-56.03 ± 0.59	-57.07 ± 3.39
DOM-pH 9-7 + MCT	-58.73 ± 1.01	-58.90 ± 1.49	-58.80 ± 1.30	-60.40 ± 2.13	-62.77 ± 1.64

Note: DOM-N = native DOM; Data are reported as mean of 6 replicates; Results are presented as mean ± standard deviation.

3.3. Emulsification properties of DOM

3.3.1. Particle diameters and stability of DOM solution

Table 2(a) shows the droplet size of DOM solutions at different concentrations. DOM solution samples tested included native DOM (DOM-N), pH-treated DOM (DOM-pH 7, DOM-pH 5, and DOM-pH 9), and DOM neutralised after pH treatment (DOM-pH 5-7 and DOM-pH 9-7). The results indicate a trend where particle size diameters increased with an increase in concentration, which may be caused by flocculation. Particle size values for the commercial emulsifier, GA at different concentrations were in the range of 0.16-0.29 μm, whereas that for native DOM ranged from 0.86 μm to 1.45 μm. Compared with that of GA (< 0.30 μm), the droplet size of DOM samples was much larger (> 0.8 μm).

The droplet diameters of DOM-N, DOM-pH 7, DOM-pH 5, and DOM-pH 9 were in the range of 0.86-1.45, 1.56-5.56, 1.34-1.59, and 0.58-1.24 μm, respectively. Although the pH value of DOM-N was 6.96 (Table 1(a)), close to pH 7.0, the droplet size of DOM-pH 7 was significantly larger than that of DOM-N. DOM-pH 7 was dialysed overnight against buffer solutions and the membrane used was 8-14 kDa. As shown in Table 1(c), since approximately 35.48% of the macromolecules within DOM measured between 10 and 15 kDa, smaller particles may have been removed during dialysis, resulting in larger droplets formed by DOM-pH 7.

The droplet diameter of DOM-pH 5 was larger than that of DOM-N, but smaller than that of DOM-pH 7. Moreover, the droplet size of DOM-pH 9 was significantly smaller than that of both DOM-N and DOM-pH 7. Both, acidic and alkaline conditions

282 resulted in smaller particle size, more so in the case of alkaline conditions. The results
283 from FTIR for amino acids showed a higher proportion of acidic groups in DOM.
284 Therefore, acidic conditions did not affect droplet size of DOM to a large extent;
285 however, alkaline conditions may have caused stereochemical reactions which altered
286 the functional groups and resulting structure of DOM.

287 After pH treatment, DOM-pH 5 and DOM-pH 9 were dialysed against several
288 changes of deionised water for 24 hrs at 4 °C until the pH value returned to 7. The
289 droplet diameter of DOM-pH 5-7 was significantly larger than that of DOM-N and
290 DOM-pH 7. Meanwhile, DOM-pH 9-7 droplet sizes reverted to that of DOM-N and
291 lower. The acidic condition may have provided additional H^+ ions, and following
292 dialysis with deionised water, smaller hydrolysed DOM particles (MW < 8 kDa) could
293 have been removed during dialysis, which may have resulted in the increase in DOM
294 particle diameter. The alkaline conditions, on the other hand, introduced additional OH^-
295 groups, which combined with dissociated H^+ ions, which in turn may have resulted in
296 a change in DOM structure, causing the polysaccharides chains to repel each other.
297 Either way, the macromolecules separated into relatively smaller structures to achieve
298 smaller particle size (Wu et al., 2015).

299 Table 2(b) shows the zeta-potential of DOM solution at different concentrations.
300 Zeta-potential is an indicator of the stabilities of emulsions. If the absolute value of
301 zeta-potential is > 30, the hydrocolloid is considered stable (Williams & Phillips, 2009).
302 The zeta-potential values of GA were close to $|\pm 30|$, while those of DOM-N samples
303 were over $|\pm 40|$, suggesting relatively good stability of DOM. Compared with the

304 zeta-potential value of DOM-N, DOM-pH 7 showed a higher value. The zeta-potential
305 values of DOM-pH 7, DOM-pH 5, and DOM-pH 9 were in the range of -57 to -47.5, -
306 37.97 to -47.37, and -44.10 to -38.83 mV, respectively.

307 The results from this study show similarity to a report by Nakauma et al. (2008),
308 who showed that a decrease in pH causes a decrease in zeta-potential. However, after
309 treatment at pH 9, the increase in pH caused a decrease in the zeta-potential in this study,
310 which contradicts the findings by Nakauma et al. (2008). Since DOM was slightly
311 acidic, more H^+ ions available in solution and zeta-potential of the original DOM
312 sample was negative. Therefore, the zeta-potential decreased slightly under acidic
313 conditions. The increase in pH provided more OH^- ions, which combined with
314 dissociated H^+ and caused the macromolecules to reconfigure their structure as the the
315 negatively charged polysaccharide chains would repel each other. Therefore, the
316 potential of pH-treated DOM caused a change in the zeta-potential.

317 After several rounds of dialysis against deionised water, the pH value of pH-treated
318 DOM samples was adjusted back to neutral. The zeta-potential values of DOM-pH 5-7
319 and DOM-pH 9-7 were in the range of -54.87 to -56.97 mV and -45.87 to -70.80 mV,
320 respectively, which were higher than that of DOM-pH 7. The results show that DOM
321 may undergo a change in structure and functional groups after pH treatment, which is
322 consistent with the results reported by Nakauma et al. (2008). Thus, the zeta-potential
323 value is not the only criterion to determine emulsion stability. According to Wu et al.
324 (2015), emulsion stability is determined by several factors including amino acid

composition, isoelectric point, and conformation of polysaccharides; an increase in polysaccharide concentration also causes an increase in stability of emulsions.

3.3.2. Emulsification properties of DOM with MCT

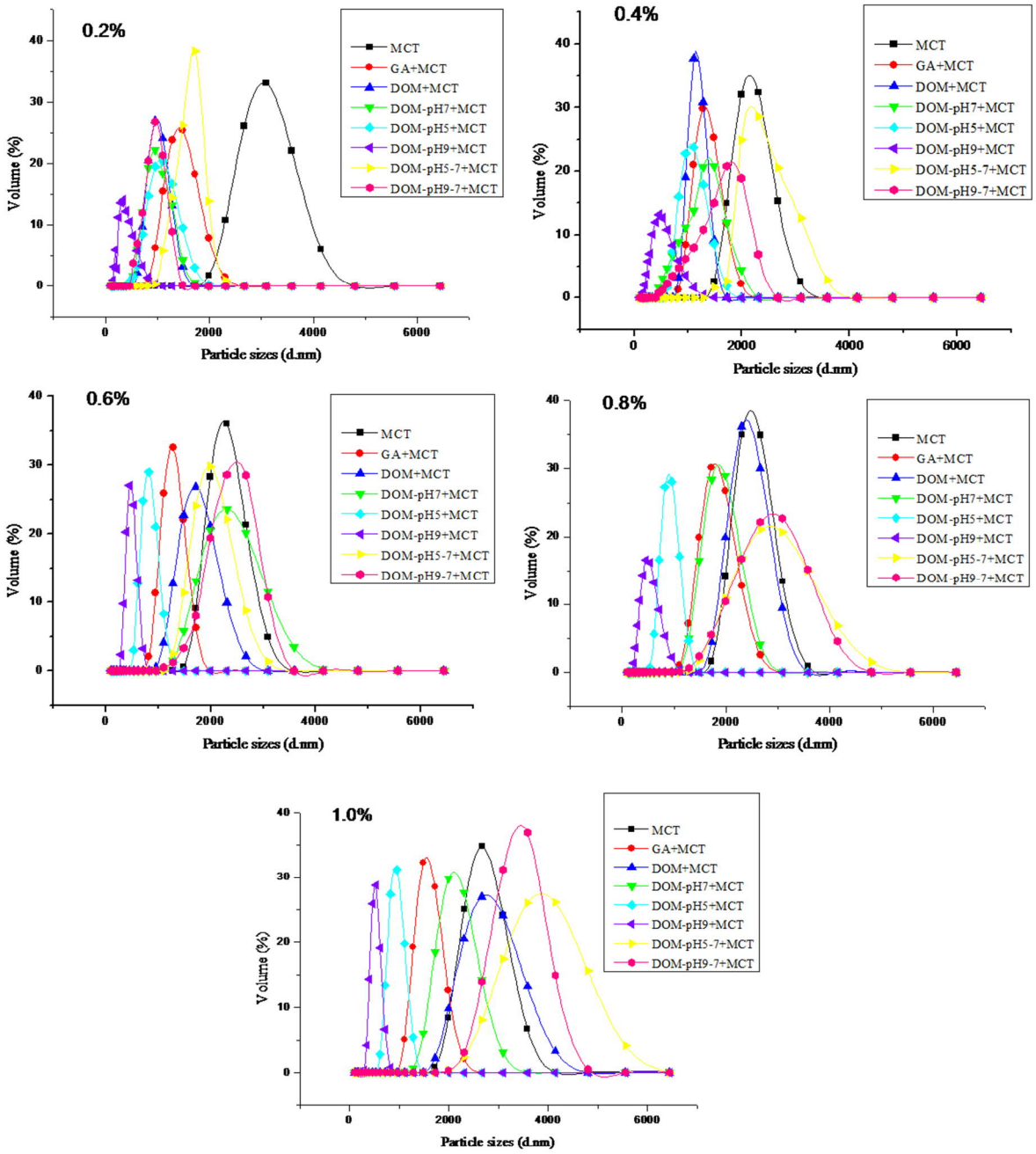
Table 3(a) shows the droplet size (z-average, μm) and PDI of emulsions stabilised by GA, DOM native (DOM-N), pH treated DOM samples (DOM-pH 7, DOM-pH 5 and DOM-pH 9), and neutralised DOM after pH treatment (DOM-pH 5-7 and DOM-pH 9-7) with MCT. The droplet size of most emulsions showed an increasing trend with an increase in concentration, with a few exceptions such as 0.8% w/v GA + MCT, 0.8% w/v DOM + MCT, 0.8% w/v DOM-pH 5 + MCT, and DOM-pH 9-7 + MCT.

The droplet sizes of MCT alone in water was in the range of 1.94 to 2.89 μm. The emulsions made from GA + MCT, and DOM + MCT (ratios = 1 : 1) showed a decrease in droplet size in the range of 1.21 to 1.78 μm, and 1.04 to 2.52 μm, respectively. The droplet size of pH-treated DOM including DOM-pH 7, DOM-pH 5, and DOM-pH 9 was in the range of 1.16 to 2.38, 0.94 to 1.16, and 0.39 to 0.54 μm, respectively. After dialysis against deionised water, molecules < 8 kDa in size passed through the membrane and therefore, the droplet sizes of DOM-pH 7 was larger than that of DOM-N. On the other hand, DOM-pH 5 showed similar/slightly smaller droplet size than DOM-pH 7, while, DOM-pH 9 showed a much smaller droplet size compared with DOM-pH 7. The results are consistent those shown in Table 2, which also suggest that OH^- ions in an alkaline aqueous solution may cause the polysaccharide chains to repel each other. Oil droplets coalesce because of the decrease in electrostatic repulsion (Wu et al., 2015). Protein in DOM contains hydrophobic groups and polysaccharides contain

347 hydrophilic groups, which repel each other. Therefore, the same amount of MCT would
348 require a lower quantity of protein and polysaccharides, which may relate to
349 conformational change or depolymerisation of the carbohydrate portion, reducing the
350 steric effect (Nakauma et al., 2008).

351 At neutralised pH, the droplet size of DOM-pH 5-7 and DOM-pH 9-7 was in the
352 range of 1.62 to 3.80 and 0.94 to 3.72 μm, respectively, which is larger than that of both
353 DOM-N and corresponding DOM-pH-treated. The results show that the pH-treated
354 DOM samples were unable to recover the emulsifying ability of DOM-N. Compared
355 with MCT alone, DOM-N exhibited better emulsification properties, indicating that
356 DOM should be investigated further as a natural unconventional food additive.

357 Table 3(b) lists the zeta-potential values of emulsions made from GA and DOM
358 samples with MCT. The zeta-potential value of each DOM sample (> 40 mV) was
359 higher than that of MCT alone as well as of emulsions made from GA and MCT
360 (approximately 30 mV). However, according to Wu et al. (2015), zeta-potential,
361 especially at different pH values, does not necessarily lead to a more stable emulsion
362 due to H^+ and OH^- ions affecting the isoelectric point. Taken together, data in Table 3(a)
363 and (b) show that mucilage obtained from *Dioscorea opposita* exhibits superior
364 emulsification properties compared with GA.



365

366 **Fig. 2.** Droplet size and distribution of freshly prepared emulsions. The ratio of GA +

367 MCT and DOM + MCT was 1 : 1 at different concentrations of 0.2%, 0.4%, 0.6%, 0.8%,

368 and 1.0% w/v. Data is presented as mean from 6 replicates.

369

Extrapolated from Table 3(a), Fig. 2 shows the droplet size distribution of emulsions stabilised by GA and DOM at different concentrations. The peaks of emulsions at 0.2% w/v concentration were tightly distributed at approximately 1,000 nm, whereas the peak for MCT (0.2% w/v) alone appears at 2,890 nm. The peaks of emulsions made from GA and MCT were quite stable, in the range of 1,210 to 1,780 nm, while those from DOM and MCT were in the range of 1,040 to 2,520 nm at different concentrations (0.2% to 1.0% w/v). The smallest droplet diameters at each concentration (0.2%, 0.4%, 0.6%, 0.8%, and 1.0% w/v) corresponded to DOM-pH 9 (390, 410, 430, 470, and 540 nm, respectively), suggesting that the increase in pH not only increased the zeta-potential value (Table 3(b)), but also lowered the droplet size. The pH 5-treated DOM also showed smaller droplet size, with diameters of 1160, 1040, 1050, 940, and 1070 nm for increasing concentrations of 0.2% through 1.0% w/v, respectively. The results indicate that DOM shows superior emulsification ability at lower concentrations, with pH 9-treated DOM showing optimum emulsifying function with small droplet size and high zeta-potential values.

4. Conclusion

This study was carried out to investigate the emulsification properties of DOM compared with GA at different concentrations and pH treatments. Large droplet diameter of DOM solution showed higher zeta-potential compared with that of GA. Emulsions made from DOM and MCT presented greater stability, especially at lower concentrations. The native pH values were 6.96 and 4.49 for DOM and GA solutions,

respectively, and both pH values of 5 and 9 showed an improvement in the overall emulsification properties. The results suggest that H^+ and OH^- ions may alter the isoelectric point of amino acids, which would cause the polysaccharide chains to repel each other. Therefore, though the zeta-potential value increased rapidly with a change in pH, the stability of the emulsion may not be affected.

In conclusion, considering the droplet size and zeta-potential value, mucilage obtained from *Dioscorea opposita* could be considered as a natural emulsifier, especially under alkaline conditions and is a sustainable resource obtained from industrial processing waste.

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