<https://helda.helsinki.fi>

Chemical components and emulsification properties of mucilage from Dioscorea opposita Thunb

Ma, Fanyi

2017-08-01

Ma , F , Zhang , Y , Yao , Y , Wen , Y , Hu , W , Zhang , J , Liu , X , Bell , A E & Tikkanen-Kaukanen , C 2017 , ' Chemical components and emulsification properties of mucilage from Dioscorea opposita Thunb ' , Food Chemistry , vol. 228 , pp. 315-322 . <https://doi.org/10.1016/j.foodchem.2017.01.151>

<http://hdl.handle.net/10138/308380>

<https://doi.org/10.1016/j.foodchem.2017.01.151>

cc_by_nc_nd

acceptedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

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

**Running Title: Characteristics and Emulsifications of Chinese yam
mucilage**

Fanyi MA^a, Yun ZHANG^a, Yanna YAO^a, Yurong WEN^a, Weiping HU^a, Mingjing LI,
Xiuhua Liu^{a*}, Alan Bell^b, Carina Tikkanen-Kaukanen^c

a. Key Laboratory of Natural Medicine and Immune-Engineering of Henan Province,
Key Laboratory for Special Functional Materials of Ministry of Education,
Pharmaceutical College, College of Chemistry and Chemical Engineering, Henan
University, Kaifeng, 475004, China.

b. Department of Food and Nutritional Science, University of Reading, Whitenights,
Reading, RG6 6AP, UK

c. Ruralia Institute, University of Helsinki, Lönnrotinkatu 7, 50100 Mikkeli, Finland

***Corresponding Author**

Prof. Xiuhua LIU,
Key Lab. of Natural Medicine and Immune-Engineering of Henan Province,
College of Chemistry and Chemical Engineering,
Henan University, Kaifeng, 475004, China.

Email: liuxiuhua@henu.edu.cn

Telephone: +86-371-22199505.

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

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

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

DOM was extracted as previously described by Andrade et al. (2015) with minor modifications. Briefly, approximately 4.0 kg fresh *Dioscorea opposita* was washed, peeled, and washed again in deionised water (pH 7.0, conductance: 18 mΩ). Approximately 300 g portions of *Dioscorea opposita* were sliced and ground in an industrial blender for 5 min. All portions were subsequently pooled and homogenised. After centrifugation at 4,000 rpm for 5 min. DOM was collected in the supernatant and freeze-dried for 3 days to a constant weight to determine DOM yield. DOM was stored in vacuum desiccators over P₂O₅ until use.

2.3. Analytical methods

2.3.1. Determination of glucose and protein content

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

2.3.2. Determination of monosaccharides

As previously described by Andrade et al., (2015), gas chromatography-mass spectrometry (GC-MS, ThermoFisher Trace 1310 ISQ) was used for the quantitative 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 ($8.0 \text{ mm} \times 300 \text{ mm}$, 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 , $50.0 \text{ }\mu\text{L}$ 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 ($1\% \text{ w/v}$) 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 $\text{m}\Omega$) 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

study. The remaining samples were then dialysed against several changes of deionised water for 24 hrs at 4 °C. No change in sample volume was observed. Materials were freeze-dried and stored in vacuum desiccators over P₂O₅ for further study.

2.4.2. Droplet distribution measurements

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

3. Results and Discussion

3.1. Components of DOM

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

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

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

Note: ND = None detected; SD = standard deviation; fucose, galacturonic acid, and glucuronic acid were tested and found below analytical detection limit.

(b) Amino acid composition, mean retention time (RT_m) and peak area of *Dioscorea opposita* mucilage

Amino Acid	Content (%)	RT _m (min)	Peak Area (×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

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

MW factors of <i>Dioscorea opposita</i> 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%

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 (RT_m) 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 $\times 7000$; (c) transmission electron microscopic image of DOM at magnifications of $\times 20,000$

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 $1395\text{-}1440\text{ cm}^{-1}$) 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).

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

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

Droplet diameter (z-average in $\mu\text{m} \pm$ standard deviation with mean PDI in parentheses)					
	Concentrations (% w/v)				
	0.2%	0.4%	0.6%	0.8%	1.0%
GA	$0.16 \pm 0.02^a(0.43)$	$0.28 \pm 0.04^{ab}(0.53)$	$0.20 \pm 0.01^{abc}(0.54)$	$0.28 \pm 0.03^{acd}(0.57)$	$0.29 \pm 0.01^{ace}(0.38)$
DOM-N	$0.86 \pm 0.06^{af}(0.56)$	$0.93 \pm 0.08^{bg}(0.57)$	$1.09 \pm 0.09^{cfgh}(0.54)$	$1.25 \pm 0.06^{dfghi}(0.39)$	$1.45 \pm 0.04^{efghij}(0.46)$
DOM-pH 7	$1.56 \pm 0.09^{afk}(0.45)$	$2.48 \pm 0.10^{bgkl}(0.47)$	$2.85 \pm 0.07^{chklm}(0.51)$	$3.23 \pm 0.06^{diklmn}(0.39)$	$5.56 \pm 0.11^{ejklmno}(0.46)$
DOM-pH 5	$1.34 \pm 0.02^{afkp}(0.39)$	$1.43 \pm 0.09^{bglq}(0.62)$	$1.44 \pm 0.02^{chmr}(0.51)$	$1.56 \pm 0.04^{dinps}(0.53)$	$1.59 \pm 0.12^{eopt}(0.36)$
DOM-pH 9	$0.58 \pm 0.02^{afkpu}(0.57)$	$0.68 \pm 0.01^{bglquv}(0.59)$	$0.85 \pm 0.03^{chmruvw}(0.56)$	$1.02 \pm 0.03^{dinsuvw}(0.54)$	$1.24 \pm 0.04^{ejotuvwxy}(0.53)$
DOM-pH 5-7	$2.46 \pm 0.10^{afkpu}(0.49)$	$3.12 \pm 0.08^{bglqv}(0.36)$	$3.18 \pm 0.07^{chmrw}(0.25)$	$4.24 \pm 0.08^{dinsx}(0.30)$	$4.85 \pm 0.37^{ejoty}(0.32)$
DOM-pH 9-7	$1.06 \pm 0.09^{afkpu}(0.22)$	$1.44 \pm 0.01^{bglv}(0.46)$	$1.53 \pm 0.05^{chmw}(0.50)$	$2.30 \pm 0.09^{dinsx}(0.34)$	$2.79 \pm 0.08^{ejoty}(0.44)$

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

251 (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

252 **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 $\mu\text{m} \pm$ 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^{ejprt} (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^{bgq} (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 ± 0.06^{bgq} (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 $\pm$ standard deviation; Paired values with
 257 superscript letters a through t indicate significant difference ($P < 0.05$).

258 (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

259 **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 ($< 0.30 \mu\text{m}$), the droplet size of DOM samples was much larger ($> 0.8 \mu\text{m}$).

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

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

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

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

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

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

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

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

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

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

Fig. 2. Droplet size and distribution of freshly prepared emulsions. The ratio of GA + MCT and DOM + MCT was 1 : 1 at different concentrations of 0.2%, 0.4%, 0.6%, 0.8%, and 1.0% w/v. Data is presented as mean from 6 replicates.

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.

Acknowledgments

We are grateful for financial support from College Science and Technology Innovation Team Program of Henan Province (No. 14IRTSTHN030), Foundation of Science and Technology Department of Henan Province (No. 152102210054), and Foundation for Young-backbone Teachers of Higher Education Institutions in Henan Province (No. 2014GGJS-023).

References

Andrade, L.A., Nunes, C.A. & Pereira, J. (2015). Relationship between the chemical components of taro rhizome mucilage and its emulsifying property. *Food Chemistry*, 178, 331-338.

Bellisle, F. (1999). Glutamate and the UMAMI taste: sensory, metabolic, nutritional

414 and behavioural considerations. A review of the literature published in last 10
 415 years. *Neuroscience and Biobehavioral Reviews*, 23(3), 423-438.

416 Bradford, M.M. (1976). A refined and sensitive method for the quantification of
 417 microgram quantities of protein utilizing the principle of protein-dye binding.
 418 *Analytical Biochemistry*, 72, 248-254.

419 Capitani, M.I., Nolasco, S.M. & Tomás, M.C. (2016). Stability of oil-in-water (O/W)
 420 emulsions with chia (*Salvia hispanica* L.) mucilage. *Food Hydrocolloids*, 61, 537-
 421 546.

422 Damodaran, S. (1996). Amino acids, peptides, and proteins. In O.R. Fennema (Ed.),
 423 *Food Chemistry* (pp. 321-429). USA: CRC Press.

424 Dickinson, E. & Stainsby, G. (1988). *Advances in Food Emulsions and Foams*. London:
 425 Elsevier Applied Science.

426 Dubois, M., Gilles, K.A., Hamilton, J.K., Reders, P.A., & Smith, F. (1956). Colorimetric
 427 method for determination of sugars and related substances. *Analytical Chemistry*,
 428 28(3), 350-356.

429 Helfman, P.M., & Bada, J.L. (1976). Aspartic acid racemisation in dentine as a measure
 430 of aging. *Nature*, 262(5566), 279-281.

431 Herlina (2015). Deproteinase effect of hydrocolloid flour made of “Gembili Tuber”
 432 (*Dioscorea esculenta* L.) on chemical and technical functional properties.
 433 *International Journal on Advanced Science, Engineering and Information*
 434 *Technology*, 5(4),298-302.

435 Huang, Z., Liang, Z., Li, G., & Hong, H. (2011). Response surface methodology to

436 extraction of *Dioscorea* polysaccharides and the effects on rat's bone quality.
 437 *Carbohydrate Polymers*, 83(1), 32-37.

438 Hsu, C.L., Chen, W., Weng, Y.M., & Tseng, C.Y. (2003). Chemical composition,
 439 physical properties and antioxidant activities of yam flours as affected by different
 440 drying methods. *Food Chemistry*, 83(1), 85-92.

441 Idris, O.H.M. & Haddad, G.M. (2012). Gum arabic's (*gum Acacia's*) journey from tree
 442 to end user. In J.F. Kennedy, G.O. Phillips & P.A. Williams (Eds.), *Gum Arabic* (pp.
 443 3-17). Croydon: The Royal Society of Chemistry.

444 Jinap, S. & Hajeb, P. (2010). Glutamate. Its applications in food and contribution to
 445 health. *Appetite*, 55(1), 1-10.

446 Kiho, T., Hara, C. & Ukai, S. (1985). A glucomannan from the tubers of *Dioscorea*
 447 *Japonica* Thunb. *Chemical & Pharmaceutical Bulletin*, 33(1), 270-275.

448 Lee, M.H., Lin, Y.S., Lin, Y.H., Hsu, F.L. & Hou, W.C. (2003). The mucilage of yam
 449 (*Dioscorea batatas* Decne) tuber exhibited angiotensin converting enzyme
 450 inhibitory activities. *Botanical Bulletin of Academia Sinica*, 44(4), 267-273.

451 Li, H., Wu, Z., Liu, W., Li, Z., Hu, N. & Huang, D. (2016). Recovery of yam mucilage
 452 from the yam starch processing wastewater by using a novel foam fractionation
 453 column. *Separation and Purification Technology*, 171, 26-33.

454 Ma, F., Bell, A.E., Davis, F.J. (2015). Effects of high-hydrostatic pressure and pH
 455 treatments on the emulsification properties of gum arabic. *Food Chemistry*, 184,
 456 114-121.

457 Masters, P.M., Bada, J.L., & Zigler JR, J.S. (1977). Aspartic acid racemisation in the
 458 human lens during aging and in cataract formation. *Nature*, 268(5615), 71-73.

459 McClements, D.J. (2005). *Food Emulsions, Principles, Practice, and Techniques*.
 460 London: CRC Press.

461 Myoda, T., Matsuda, Y., Suzuki, T., Natagawa, T., Nagai, T. & Nagashima, T. (2006).
 462 Identification of soluble proteins and interaction with mannan in mucilage of
 463 *Dioscorea opposita* Thunb. (Chinese yam tuber). *Food Science and Technology*
 464 *Research*, 12(4), 299-302.

465 Nakauma, M., Funami, T., Noda, S., Ishihara, S., Al-Assaf, S., Nishinari, K. & Phillips,
 466 G.O. (2008). Comparison of sugar beet pectin, soybean soluble polysaccharide,
 467 and gum arabic as food emulsifier. 1. Effect of concentration, pH, and salts on the
 468 emulsifying properties. *Food Hydrocolloids*, 22(7), 1254-1267.

469 Nep, E.I. & Conway, B.R. (2010). Characterization of grewia gum, a potential
 470 pharmaceutical excipient. *Journal of Excipients and Food Chemicals*, 1(1), 30-40.

471 Ohtani, K. & Murakami, K. (1991). Structure of mannan fractionated from water-
 472 soluble mucilage of nagaimo (*Dioscorea batatas*Dence). *Agricultural and*
 473 *Biological Chemistry*, 55(9), 2413-2414.

474 Télessy, I.G., Balogh, J., Csempesz, F., Szente, V., Dredán, J. & Zelkó, R. (2009),
 475 Comparison of the physicochemical properties of MCT-containing fat emulsions
 476 in total nutrient admixtures. *Colloids and Surfaces B: Biointerfaces*, 72(1), 75-79.

477 Xue, Y., Miyakawa, T., Nakamura, A., Hatano, K., Sawano, Y. & Tanokura, M. (2015).
 478 Yam tuber storage protein reduces plant oxidants using the coupled reactions as

479 carbonic anhydrase and dehydroascorbate reductase. *Molecular Plant*, 8(7), 1115-
 480 1118.

481 Waqas, M., Khan, A.L., Hamayun, M., Shahzad, R., Kim, Y.H. & Choi, I.J. (2015).
 482 Endophytic infection alleviates biotic stress in sunflower through regulation of
 483 defence hormones, antioxidants and functional amino acids. *European Journal of*
 484 *Plant Pathology*, 141(4), 803-824.

485 Williams, P.A. & Phillips, G.O. (2009). *Handbook of Hydrocolloids*, 2nd Edition. Oxford:
 486 Woodhead Publishing Limited.

487 Wu, Y., Eskin, N.A.M., Cui, W. & Pokharel, B. (2015). Emulsifying properties of water
 488 soluble yellow mustard mucilage: A comparative study with gum arabic and citrus
 489 pectin. *Food Hydrocolloids*, 47, 191-196.

490 Yang, D. & Lin, J. (2008). Effects of different storage conditions on steroidal saponins
 491 in yam (*Dioscorea pseudojaponica* Yamamoto) tubers. *Food Chemistry*, 110(3),
 492 670-667.

493 Zeng, W. & Lai, L. (2016). Characterization of the mucilage extracted from the edible
 494 fronds of bird's nest fern (*Asplenium australasicum*) with enzymatic modifications.
 495 *Food Hydrocolloids*, 53, 84-92.

496 Zhang, Z., Wang, X., Liu, C. & Li, J. (2016). The degradation, antioxidant and
 497 antimutagenic activity of mucilage polysaccharide from *Dioscorea opposita*.
 498 *Carbohydrate Polymers*, 150, 227-231.