Extraction and characteristic of *Dioscorea alata* mucilage

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**Abstract.** *Dioscorea alata* (DA) mucilage is classified as water-soluble polysaccharides, which has potential as a source of hydrocolloid. This research was conducted to investigate effects of salt types on water to tuber ratio to produce the most optimal mucilage yield. This research was conducted using Completely Randomized Factorial Design 3 x 5. The DA mucilage was extracted using two factors, i.e., types of salt (without salt, sodium chloride, and calcium chloride) and water to tuber ratios (2:1, 4:1, 6:1, 8:1, and 10:1). The results showed that the types of salt significantly affect (*p*<0.05) on starch content, but did not have a significant effect on mucilage yield and water content of the mucilage. The water to tuber ratios significantly affected (*p*<0.05) the mucilage yield and starch content of the mucilage, however it did not affect the water content. Addition of calcium chloride when water to tuber ratio was 4:1 was recommended to produce the optimal mucilage yield (1.58%) with relatively low starch content (7.86%).

1. Introduction

Hydrocolloids are hydrophilic polymers derived from various sources such as plants, animals, microorganisms or chemical modification, that have multi functionalities such as thickener, gelling agent, stabilizer, etc. The world market of hydrocolloids was estimated to reach USD 8.77 billion (around IDR 119.9 trillion) in 2018, but price instability and shortage of raw materials were considered to be obstacles in hydrocolloids market [1].

*Dioscorea* sp. is one of the tubers belongs to Dioscoreaceae family that has a potential as hydrocolloids source because of its mucilage content. The viscous mucilage mainly contains protein and polysaccharides in the form of glycoprotein, a mannan that is firmly associated with protein [2]. *Dioscorea* mucilage is water soluble carbohydrates-protein complexes with a ratio between soluble carbohydrates and protein of 1:8.1. Monosaccharide composition in mucilage of *Dioscorea alata* (DA) mainly composed of mannose (95.4%). Other units like glucose, galactose, arabinose, xylose, and rhamnose also present at low quantities, i.e., below 5% [3]. A previous research showed that mucilage yield extracted from DA is higher than that of taro and sweet potato. On dry basis, DA produced 6.51 g of mucilage (/100 g material) compared to 3.23 g per 100 g taro and 5.12 g per 100 g sweet potato [4]. Rehydrated mucilage of DA can be used to produce oil-in-water or air-in-water emulsion (foam) even at concentration of 0.1% with good stability. Emulsions with 0.5% DA mucilage was reported to have soft creamy and thixotropic semisolid texture [5]. In addition, DA
mucilage has antioxidant, antiradical, and immunostimulatory activity. DA mucilage var. purpurea (Roxb.) Ming-Jen has an inhibition concentration (IC50) of 0.547 mg/ml [6,7].

Several studies on mucilage extraction from Dioscorea sp. have been done using water as the solvent. Ratio of water to solid material is one of the main factors during extraction [6]. Some studies used water to solid ratios that vary from 0: 1 to 10: 1 [9,10]. The mucilage yield increased with increasing water to solid ratio before it turned to be constant and then, decreased at a certain point. The ratio of water to solid plays an important role in reducing resistance to mass transfer during extraction. Enzymes can be activated to facilitate separation of protein and starch from the mucilage by increasing water to solid ratio. However, the optimal ratio must be defined to minimize extraction cost [10].

One of the challenges in extracting mucilage from DA is high viscosity and high water-binding capacity of glycoprotein that inhibit separation of mucilage from starch [3]. Salts have been widely used to release mucilage during starch extraction from various plants [10]. Sodium chloride (NaCl) at a concentration of 1% was used to remove mucilage during starch extraction of Dioscorea tuber [11]. Addition of 0.3 M NaCl was reported to be effective in removing mucilage from taro tuber [12]. The addition of NaCl with concentrations of more than 0.5% reduces water holding capacity; therefore, mucilage would be easier extracted from the tubers [13].

This research was performed to investigate effects of salt types on water to tuber ratio during mucilage extraction from Dioscorea alata tuber to produce the most optimum mucilage yield. The experiments were carried out using two salts commonly used in food industry, i.e., NaCl (monovalent salt), and calcium chloride (CaCl2, divalent salt).

2. Materials and methods

2.1 Materials and chemicals

Dioscorea alata (yellow yam) was harvested from one of the farming complexes within Bogor Agricultural University. The tubers were harvested after their stems withered, which approximately 12 months after planting.

Analytical grade reagents used in this study were absolute ethanol (Cat. No. 1009835000, Merck, Germany), NaCl (Cat. No. 1064041000, Merck, Germany), CaCl2 (Cat. No. 1023780500, Merck, Germany) and distilled water.

2.2 Extraction of mucilage from Dioscorea alata tuber.

Fresh tubers were washed, peeled, and sliced around 1 cm thickness. Sliced tubers (250 g) were steam blanched for 10 min and further blended for 10 min in 1% w/w of salt solutions, with different water to tuber ratios. The slurry was stored at 10°C for 24 hours and then, filtered through a 105 µm sieve. Further, the filtrate was centrifuged (Sorvall™ Legend™ XTR centrifuge, Fisher Scientific Ltd, UK) at 12,000g for 20 min at 4°C from which the supernatant was collected. The supernatant was mixed with ethanol with a ratio of 1:1 (v/v) and kept at 4°C for 24 hours and then, centrifuged at 12,000g for 20 min at 4°C to precipitate the mucilage. The precipitate was freeze-dried and then, ground to obtained mucilage powder. The powder was stored at room temperature for further analysis.

The experiments in this research was a 3 × 5 factorial in Completely Randomized Design. The independent variables were three types of salt (control/water, NaCl, and CaCl2) and five of water to tuber ratios (2:1, 4:1, 6:1, 8:1, and 10:1). The experiments were carried out in duplicates. The data were evaluated for their significant differences by Duncan’s Multiple Range Test (DMRT) (p = 0.05).

2.3 Chemical composition of fresh Dioscorea alata tubers

The moisture and crude ash were determined using gravimetric method, crude fat content was analysed using soxhlet extraction method, crude protein content was measured using Kjeldahl method, and carbohydrate content was calculated by difference method [3,8]. Starch content was determined using Luff Schoorl method. Each method of analysis was performed in triplicate.

2.4 The mucilage yield

The mucilage yield was expressed as ratio (% w/w, YM) between the weight of extracted mucilage (A) and the initial weight of fresh tuber (B) [14], as shown in equation (1).
2.5 Starch content of the mucilage

The starch content of DA mucilage was determined using Megazyme’s Total Starch (α-amylase/Amyloglucosidase, AA/AMG) Assay kit (K-TSTA) [8]. This analysis was performed to check purity of the mucilage.

2.6 Mucilage morphology

The morphology of dried mucilage was observed using an environmental scanning electron microscope (SEM) (FEI Quanta 650, FEI Company, Hillsboro, Oregon, USA). The samples were placed on carbon conductive tape without coating. Then, the scanning was operated at low vacuum mode using a Large Field Detector (LFD) at 500× magnification, 10 mm of working distance, with pressure, voltage and temperature of 60 Pa, 10 kV, and 20°C respectively.

3. Results and discussion

3.1 Chemical composition of fresh Dioscorea alata tubers

Chemical composition of the fresh tubers (without peel) is stated in table 1. On a wet basis, moisture was the main content of fresh tubers. On a dry basis, the highest content was carbohydrate, followed by starch and then, protein. The values presented in table 1 are in accordance with previous studies that reported varying chemical contents of tubers, i.e., 15.24 – 70.88% of carbohydrate, 14.25 – 77.40% of starch, and 2.86 – 13.20% of protein [9,10,15,16]. These results confirm previous researches that DA mucilage mainly contains carbohydrates and protein. This composition might lead to high viscous slurry that makes separation process becomes challenging.

| Parameter       | % dry basis | % wet basis |
|-----------------|-------------|-------------|
| Moisture        | -           | 61.65       |
| Crude Ash       | 2.76        | 1.06        |
| Crude Fat       | 0.31        | 0.12        |
| Protein         | 32.41       | 12.43       |
| Carbohydrates   | 64.51       | 24.74       |
| Starch          | 44.82       | 17.19       |

3.2 Mucilage yield

This research indicated that types of salt did not significantly affect the mucilage yields (p>0.05), as shown in table 2. On the other hand, water to tuber ratios significantly affected the yields (p>0.05). There were no interaction effects between types of salt and water to tuber ratios. The result shows that the mucilage yield increased with increasing water to tuber ratios, and the highest yield was obtained when water to tuber ratio was 6:1. Increasing the ratio beyond this resulted in decreasing mucilage yield. Nevertheless, this research resulted in 10× higher mucilage yield than a previous study [9] at the highest water to tuber ratio (10:1). This might be attributed to centrifugational process of filtrate during separation.

The mucilage yield increased with increasing ratio of water to tuber. At high water to tuber ratio, more volume of water is available to penetrate and diffuse through the tuber matrices. More water presents would bind more water-soluble compounds in the tuber cells, including the mucilage, and they are easier to be extracted from the cells. A higher volume of water presents during extraction would also make less viscous slurry, therefore, separation of water-soluble compounds from the tubers is more efficient. This particular results are in line with previous studies that reported a higher yield of mucilage extracted from various Dioscorea sp. [17,18,19].

| The type of salts | Mucilage yield (%) |
|------------------|---------------------|

Water and NaCl

The water to tuber ratio

| Water to Tuber Ratio | Mucilage Yield (%) |
|---------------------|--------------------|
| 2:1                 | 0.58^a             |
| 4:1                 | 1.58^{bc}          |
| 6:1                 | 2.06^c             |
| 8:1                 | 1.46^b             |
| 10:1                | 1.40^b             |

Means with the same superscript in the same column are not significantly different (p>0.05).

Salt addition was expected to have positive effects in the mucilage extraction from the tubers, which is not significantly observed in table 2. The addition of salt causes osmotic pressure, which tends to push water together with all water-soluble compounds out of the cells. The addition of NaCl also causes an increase in ionic strength, affects hydrogen bonds and ionic bonds, changes the higher-order structure of mucilage; therefore, the mucilage viscosity decreases due to increased surface hydrophobicity [19]. Another author described that the apparent viscosity of *Mesona blues* gum is lower with addition of CaCl\textsubscript{2} than NaCl [20]. This was also inline with another study on viscosity of *Dicerocaryum zanguebarium* mucilage that decreased steadily when CaCl\textsubscript{2} or NaCl was added [20]. Nevertheless, salts can be attributed to the loss of the hydrophilic–hydrophobic balance of the polymer networks [21], which might affect water holding capacity of polysaccharides [22]. This might be the reason for insignificant effects of salt addition to the mucilage yields as shown in table 2.

### 3.3 Starch content of mucilage

Table 3 shows that water to tuber ratios, types of salt, and their interactions had significant effects (p>0.05) on starch content in the mucilage. Different water to tuber ratios did not significantly affect the starch content in the mucilage when water did not contain any salts. When either NaCl or CaCl\textsubscript{2} was added, the results can be devided into two groups of starch content. The group with low starch content was attributed to water to tuber ratio of 2:1 and 4:1; meanwhile, the group with high starch content was attributed to water to tuber ratios higher than 4:1. This research aimed for as low starch content in the mucilage as possible, although the presence of starch in the mucilage was inevitable. Overall, starch contents in the mucilage obtained in this research were much lower that the value reported previously by Alves et al. [9] which was about 43%.

**Table 3.** Starch content (%) of mucilage extracted with various water to tuber ratios and different salts.

| Water to Tuber Ratio | Types of Salt | Water | NaCl | CaCl\textsubscript{2} |
|---------------------|---------------|-------|------|---------------------|
| 2:1                 |               | 16.99^a\textsubscript{A} | 14.04^a\textsubscript{A} | 4.42\textsuperscript{b}^\textsubscript{A} |
| 4:1                 |               | 17.40^a\textsubscript{A} | 17.85^a\textsubscript{AB} | 7.86\textsuperscript{b}^\textsubscript{A} |
| 6:1                 |               | 19.64^a\textsubscript{A} | 18.78\textsuperscript{AB} | 16.62\textsuperscript{a}^\textsubscript{B} |
| 8:1                 |               | 19.79^a\textsubscript{A} | 18.18\textsuperscript{AB} | 15.44\textsuperscript{a}^\textsubscript{B} |
| 10:1                |               | 18.77^a\textsubscript{A} | 18.68\textsuperscript{AB} | 14.54\textsuperscript{AB} |

Means followed by the same letter do not significantly differ by Duncan’s test (p>0.05).

\textsuperscript{AB}Values with different superscripts within the same column significantly different (p<0.05).

\textsuperscript{a}Values with different superscripts within the same row significantly different (p<0.05).

The mucilage with the lowest starch content was produced using water to tuber ratio lower than 4:1 added with CaCl\textsubscript{2}(table 3). It is still not clear from the results how salts affected starch content in the mucilage, especially with low ratio of water to tuber. However, it has been known that salts encourage breakdown of starch, i.e., depolymerization of glucose chains into smaller molecules [23] and affect starch gelatinization. When starch is gelatinized, other molecules such as proteins are easier to be
extracted. The gelatinization inhibition effect of Ca$^{2+}$ is lower than that of Na$^{+}$ with concentrations of less than 1 M. Protein solubility can also increase when Ca$^{2+}$ presents rather than Na$^{+}$ [24]. These might lead more effective separation process, therefore, higher purity of mucilage.

3.4 Mucilage morphology

Figure 1 and Figure 2 show morphologies of DA mucilage powder captured using SEM at 500× and 5000× magnification, respectively. SEM was used to obtain three-dimensional information on the effects of the types of salt on surface morphology of DA mucilage particles. The surface morphology and structure of mucilage particles are affected by extraction, purification, and preparation methods [30]. This research shows that types of salt affected porosity of DA mucilage particles. Without salt, particles of DA mucilage were fragmented with slightly porous surface and irregular shape (Figure 1a and Figure 2a). Particles of DA mucilage with NaCl salt were dense with smooth surface (Figure 1b and Figure 2b). Last, addition of CaCl$_2$ during mucilage extraction produced mucilage particles with porous structure (Figure 1c and Figure 2c). A research indicated that high concentration of salts affects starch gelatinization and surface morphology of the starch from various tubers [31]. However, further studies are required to explain salt effects on morphology of dried mucilage.

![Figure 1](image1)

**Figure 1.** Scanning electron microscope image of *Discorea alata* mucilage (The water to tuber ratio of 6:1) at magnifications of 500x (a) without salt, (b) NaCl, (c) CaCl$_2$

![Figure 2](image2)

**Figure 2.** Scanning electron microscope image of *Discorea alata* mucilage (The water to tuber ratio of 6:1) at magnifications of 5000x (a) without salt, (b) NaCl, and (c) CaCl$_2$

4. Conclusions

This research shows that water to tuber ratios used during extraction of DA mucilage affected the yield. The ratio together with addition of salt affected starch content as well as morphology of mucilage particles. Water to tuber ratio of 4:1 with addition of CaCl$_2$ salt resulted in mucilage with the optimal yield (1.58%) and high purity (low starch content). When NaCl was added, water to tuber ratio of 6:1 must be applied to get the highest yield of mucilage (2.06%).

Acknowledgments

The research was financially supported by Beasiswa Unggulan Dosen Indonesia Dalam Negeri (BUDI-DN), The Indonesia Endowment Fund for Education (LPDP), Ministry of Finance Republic of Indonesia, and Ministry of Research, Technology and Higher Education of the Republic of Indonesia.
References

[1] Research GV. San Fransisco; 2019. Available from: https://www.grandviewresearch.com/industry-analysis/hydrocolloids-market
[2] Misaki A, Ito T, and Harada T 1972 Agric Biol Chem 36 761
[3] Fu YC, Chen S, and Lai YJ 2004 J Food Sci 69 509
[4] Huang C, Lai P, Chen I-H, Liu Y, and Wang C-CR 2010 LWT - Food Sci Technol 43 849
[5] Tingjiang L, YawMing L, and Huelju W 2012 Taiwan J Agric Chem Food Sci 50 263
[6] Shang H-F, Cheng H-C, Liang H-J, Liu H-Y, Liu S-Y, and Hou W-C 2007 Bot Stud 48 63
[7] Lin S, Liu H, Lu Y, Hou W 2005 Bot Bull Acad Sin 46 183
[8] Fu Y-C, Chen S-H, Huang P-Y, and Li Y-J J 2005 Agric Food Chem 53 2392
[9] Alves RM, Grossmann MV, Ferrero C, Zaritzky NE, Martino MN, and Sierakoski MR 2002 Starch/Staerke 54 476
[10] Gidley MJ, Reid JSG. In: Food Polysaccharides and Their Applications. 2006. p. 181–215.
[11] Riley CK, and Adebayo SA. 2006 Starch - Stärke 58 418
[12] Widowati S, Waha M., Santos BA. In: Seminar Teknologi Pangan, Book 1. Jakarta: Multi Pangan Selina; 1997. p. 181–95.
[13] Hernández LM. Pontifica Universidad Catolica de Chile; 2012.
[14] Jayakody L, and Hoover R, Q L, E D. 2007 Carbohyr Polym. 69 148
[15] Oko AO, and Famurewa AC. Br 2015 J Appl Sci Technol 6 145
[16] Hong-rui L, Shu-Lin Y, Yun-chao C, and Li-qiang Z. 2010 Med Plant 1 29
[17] Luo D 2012 Carbohyr Polym 90 284
[18] Hou WC, Hsu FL, and Lee MH 2002 Planta Med 68 1072
[19] Nagashima T, Tsukui M, Sato H, and Kozima TT 2000 Journal of Japan Conversation Science. 26 3
[20] Feng T, Gu ZB, Jin ZY 2007 Food Sci Technol Int 13 55
[21] Saklani S, Chandra S, and Mishra AP 2014 Int J Pharm Sci Rev Res 23 42
[22] Mateos MP, Montero P. 2002 Food Hydrocoll 16 363
[23] Moreau L, Bindzus W, Hill S. 2011 Starch - Stärke 63 676
[24] Jane J, IA A. 1993 Starch - Stärke 45 161
[25] Nep EI, and Conway BR 2011 Carbohyr Polym 84 446
[26] Koch K, and Jane J-L 2000 Cereal Chem 77(2) 115