Oligosaccharides Profile and Prebiotic Potential of Gembolo Tuber (Dioscorea bulbifera)

E R N Herawati1*, M Miftakhussolikhah1, R Nurhayati1, K W Sari2 and Y Pranoto2

1 Research Unit for Natural Product Technology, Indonesian Institute of Sciences, Jl. Jogja-Wonosari km 31,5 Gading, Playen, Gunungkidul, Yogyakarta- 55861, Indonesia
2 Faculty of Agricultural Technology, Gadjah Mada University, Jln. Sosio-Yustisia, Bulaksumur, Yogyakarta, Indonesia

Email: ervika.rnh@gmail.com

Abstract. Indonesia has many potential tubers that not yet utilized, whereas the tubers can be utilized in functional food. Dioscorea bulbifera contains oligosaccharides which can be used as functional food prebiotics based. This research aim was to analyze the content of oligosaccharides from Dioscorea bulbifera tuber flour and its prebiotics potential. The extraction of oligosaccharides from Dioscorea bulbifera flour was done using ethanol 70% and analyze the content of oligosaccharides using HPLC (High-Performance Liquid Chromatography). The inoculum of colonic microbial used from volunteer infants aged less than 6 months and only consume breast milk. The prebiotic index was determined by enumerating the growth of total bacteria, microbial probiotics (Bifidobacteria and Lactobacillus) and microbial pathogens (Bacteroids and Clostridium) in anaerobic conditions at 0, 24 and 48 hours. The results showed that oligosaccharides profile of Dioscorea bulbifera flour were Inulin 0.76; Raffinose 0.07; and Lactulose 0.09% b/v. The prebiotics index value of oligosaccharide of extracts of Dioscorea bulbifera flour at 48th hour is 1.2045. It can be concluded that the oligosaccharide from Dioscorea bulbifera tuber flour has potential as a prebiotic.

Keywords: functional food, Dioscorea bulbifera, oligosaccharides, prebiotic, probiotic

1. Introduction

Currently, food not only serves to meet the needs but also to maintain health known as functional food. Functional food could be described as any food or ingredient that may provide a health benefit beyond the traditional functions hitherto known[1]. One of the food ingredient that beneficially affects the body is prebiotic. In recent years, increasing attention has been focussed on the possible beneficial effects of prebiotics [2]. Prebiotics has been proved for their effect in increasing absorption and bioavailability of calcium, magnesium, zinc, and iron [3]. Prebiotics are selectively fermented materials that cause specific changes in both the composition and / or microbiota activity in the colon that provide benefits to host health [4]. Prebiotics are generally carbohydrates (poly- and oligosaccharides) that can not be digested in the host channel. Some types of oligosaccharides that have been known as prebiotics are
fructooligosaccharides (FOS), glucooligosaccharides (GOS), inulin, and Raffinose. Oligosaccharides are commonly found in grains, beans, and tubers. Gembolo (Dioscorea bulbifera L.) is a kind of tubers from Dioscoreaceae family that grows much as wild plants in Indonesia but not yet widely used.

Many researchers have investigated the medical potency of gembolo. Gembolo, which rich in diosgenin, a steroid saponin which believed to possess preventive and therapeutic properties against several ailments including arthritis, cancer, diabetes, gastrointestinal disorders, high cholesterol and inflammation [5]. Copper nanoparticles synthesized by Dioscorea bulbifera has α-amylase and α-glucosidase inhibitory activity [6]. Gembolo tubers contain high carbohydrates (19.8%), and glucomannan as the main polysaccharide content. Among the carbohydrate content, the oligosaccharide compound in the gembolo tuber is potentially prebiotic. At the moments, there is little data research on the oligosaccharide profile and prebiotic potential of gembolo tuber. Therefore, this research aim was to analyze the content of oligosaccharides from Dioscorea bulbifera tuber flour and its prebiotics potential.

2. Methodology

2.1. Materials

The material used was gembolo (Dioscorea bulbifera) tuber from local area (Gunungkidul, Yogyakarta), 70% ethanol, sugar standard (raffinose, inulin, lactulose, glucose, galactose, and fructose), feces from baby volunteers younger than 6 months and still consuming breast milk, Phosphate Buffer Saline (Sigma), basal media, CO₂ gas, media plate count agar (Oxoid), agar Columbia media (Oxoid), rogossa agar medium (Oxoid), reinforced clostridial agar medium (Oxoid), tryptone soya agar medium (glucose), glucose, heamin (Sigma), glacial acetic acid (Merck), L-cysteine HCl (Merck), lached horse blood (Oxoid), brucella selective supplement (Sigma), antibiotics include kanamycin, vancomycin, and colistin (Sigma). The tools used were a grinder, sieve, oven (Memmert), Soxhlet tools, rotary evaporator, HPLC (High-Performance Liquid Chromatography), magnetic stirrer, hand counter, hot plate (Thermo scientific), autoclave (TOMY SX-700), pH meter.

Research methodologies consisted of (i) production of gembolo flour; (ii) extraction and analysis oligosaccharides profile of gembolo flour; and (iii) characterization prebiotic potency and determine prebiotic index.

2.2. Production of Gembolo flour

Making of gembolo flour consisted of some procedure: peeled Gembolo tuber skin and washed with clean water, sliced Gembolo in small part, dried with an oven at 50-60 °C, then ground using a grinder and sieved using a 60 mesh sieve to obtain the Gembolo flour.

2.3. Extraction and analysis oligosaccharides profile of Gembolo flour

The oligosaccharide extraction of Gembolo powder refers to reference [7]. Analysis of total dissolved solids in Gembolo extract was referred to as reference [8]. Analysis oligosaccharides profile of gembolo flour using HPLC method [9]. The column used was Metacharb 87 ºC with RID detector, eluent H₂O, the flow rate of 0.6 ml/min and temperature about 85 ºC. Sugar standards used were inulin, raffinose, lactulose, glucose, galactose, and fructose with each concentration was 2500 ppm. The sample was centrifuged at 12000 rpm for 5 minutes. Then diluted 5 times with aquabidest and filtered with millex 0.45 µm. A sample of 20 µl was injected into HPLC.

2.4. Characterization prebiotic potency and determine prebiotic index

2.4.1. Preparation of fecal fluid

Analysis of prebiotic potency initiated with the preparation of colonic microbes. Fecal fluid preparation refers to the reference [10]. Feces samples obtained from infants younger than 6 months and consuming only breast milk were collected and dissolved in 0.1 M phosphate buffer saline (PBS) sterile, pH 7 with a
ratio of 1:10 (w/v). Then homogenized with a magnetic stirrer (200 rpm, 2 min). The liquid at the top of the precipitate was taken and used as an inoculum for further testing.

2.4.2. Preparation of colonic microbes
Preparation of the culture of colonic microbial was referred to the reference [10], incubated at 37 ºC and samples taken at certain time intervals (0, 24, and 48 hours). Parameters analyzed for each sampling were bacterial growth, prebiotic index, and pH decreased. Isolation and analysis of total growth of bacteria, microbial probiotics (Bifidobacteria and Lactobacillus) and microbial pathogens (Clostridium and Bacteroides) referred to the reference [11-12] with modification. Total bacteria isolation was done using plate count agar. Isolation of microbial probiotics (Bifidobacteria) was done using Columbia agar medium supplemented with glucose (5g/l), L-cysteine.HCl (0.5 g/l), propionic acid (0.5 ml/m), or adjusted to pH 5 and isolation Lactobacilli microbial probiotics use rogosa agar supplemented with glacial acetic acid (1.32 ml/l). The medium used for microbial isolation of Clostridium pathogens was reinforced clostridial agar supplemented with vancomycin (8 mg/l), colistin (8 mg/l). Isolation of Bacteroides pathogenic microbials was done using tryptone soya supplemented with brucella selective supplement (5 ml/l), kanamycin 975 mg/l, haemin (5 mg/l), vancomycin (75 mg/l), laced horse blood (50 ml/l). Enumeration of the total microbial colon then calculated by measuring the growth of Total Bacteria, Bifidobacteria, Lactobacillus, Clostridium, and Bacteroides. Each time interval of the 0, 24, and 48 hours was done to sampling the colonic microbial culture. Prebiotic Index was a measure to the reference [13]. Prebiotic index of oligosaccharide from Gembolo can be determined by using the equation:

\[
\text{PI} = \frac{\text{Bif}}{\text{Total}} - \frac{\text{Bac}}{\text{Total}} + \frac{\text{Lac}}{\text{Total}} - \frac{\text{Clos}}{\text{Total}}
\]

Where: Bif is the amount of Bifidobacteria, Bac is the amount of Bacteroides, Lac is the amount of Lactobacilli, and Clos is the amount of Clostridia.

The sampling time used were 0, 24 and 48 hours. In this equation, it is assumed that the population increase of Bifidobacteria and / or Lactobacilli is a positive effect while the increase of Bacteroides and Clostridia is negatively affected. Data analysis was done by analyzing the obtained data and present it in the form of tables and graphs.

3. Results and discussion

3.1. Analysis Oligosaccharide Profile of Gembolo Flour
Analysis of oligosaccharide content in Gembolo flour extract was performed using High-Performance Liquid Chromatography (HPLC). HPLC test results for the analysis of oligosaccharide content of flour Gembolo can be seen in Table 1.

| Parameter | (% b/v) |
|-----------|---------|
| Inulin    | 0.76    |
| Raffinose | 0.07    |
| Lactulose | 0.09    |
| Galactose | 0.22    |
| Glucose   | 2.53    |
| Fructose  | 2.98    |

As shown in Table 1, the highest oligosaccharide content in Gembolo extract was inulin which contains about 0.76%. Inulin is a polysaccharide consisting of fructose units with a glycosidic bond β- (2-1) and a glucose terminal at the end [14]. Inulin is not digested by enzymes in the small intestine due to
the presence of the β-(2,1) linkages, contributing to its functional properties as prebiotic [15]. Inulin and galactooligosaccharides (GOS) are two major carbohydrates that fulfill the prebiotics criteria [16]. Many research report about the prebiotic activity of inulin from tuber [17-20]. The high content of inulin in the Gembolo extract shows the potential of prebiotics. The lowest oligosaccharide content in Gembolo was raffinose which contains about 0.07%. Raffinose is a disaccharide consisting of glucose, galactose and fructose monomers. Raffinose can be metabolized by intestinal microflora resulting in lactic acid, acetic acid, butyric acid, hydrogen peroxide, bacteriocin and other metabolites [21]. Raffinose can stimulate the growth of Bifidobacteria. Raffinose consumption in humans about 15 g/day can significantly increase the number of fecal Bifidobacteria and decrease the amount of Clostridium sp. and Bacteroides due to a decrease in fecal pH. As described in Table 1, the largest monosaccharide content was fructose which contains about 2.98%. The fructose content may show the inulin content present in the extract because inulin is a polymer of the fructose units associated with the β- (2-1) glycosidic bond with the glucose terminal group. The content of glucose and galactose in Gembolo tuber was 2.53 and 0.22%.

3.2. Prebiotic Analysis of Gembolo Flour Oligosaccharide
The growth of microbial on the media added gembolo flour oligosaccharide extract could be seen in Table 2.

| Observation time (h) | Log Cfu/ml |
|----------------------|------------|
|                      | Lactobacillus | Bifidobacteria | Bacteroides | Clostridium | Total Bacteria |
| 0                    | 3.46        | 6.95          | 1.30        | 4.52        | 5.62          |
| 24                   | 4.88        | 7.35          | 1.89        | 5.25        | 6.90          |
| 48                   | 6.03        | 7.44          | 2.84        | 4.57        | 6.84          |

As shown in Table 2, at 0 hour, the total bacteria amount was 5.62 log cfu/ml, then increased at 24 hours to 6.90 log cfu/ml, and at 48 hours it decreased to 6.84 log cfu/ml. Total bacteria are the total amount of microbes present in the sample, so the amount should be higher than the number of other microbes. Increasing the total number of bacteria indicates that microbes are able to grow on a medium containing Gembolo oligosaccharide extract. At 0 hour, the number of Lactobacillus was 3.46 log cfu/ml, then at the 24th hour it increased to 4.88 log cfu/ml, and at the 48th hour, it increased to 6.03 log cfu/ml. The presence of oligosaccharides in the media causes Lactobacillus growth well. At 0 hour, the number of Bifidobacteria was 6.95 log cfu/ml, then at 24 hours it increased to 7.35 log cfu/ml, and at 48 hours it increased slightly to 7.44 log cfu/ml. Oligosaccharide present in the media is utilized by Bifidobacteria to metabolism and growth well. Colonic microbes, such as Lactobacillus and Bifidobacteria, have increased in each time interval of observation. This suggests that the microbial colon is able to grow on a medium containing Gembolo oligosaccharide extracts. Oligosaccharides stimulate the growth of colonic microbes in the presence of inulin and glucose. Glucose is included in the monosaccharide group, so bacteria can directly use it without having to chop it first into smaller monomers.

At the 0 hour, the number of Bacteroides was 1.30 log cfu/ml, then at 24 hours it increased to 1.89 log cfu/ml, and at 48 hours it increased to 2.84 log cfu/ml. The increasing number of Bacteroides at 24 hours may be due to the pH of the medium that is still the pH optimum for Bacteroides growth. At first sampling, the Clostridium count was 4.52 log cfu/ml; then at 24 hours, it increased to 5.25 log cfu/ml and the 48th hour it slightly decreased to 4.57 log cfu/ml. The increasing number of Clostridium in the 24th hour can be due to the high glucose content in the medium can still be utilized by Clostridium to
metabolism and growth. Pathogenic microbes, such as Bacteroides, increase in the 24th hour and 48th time, unlike Clostridium which decreases at 48 hours. This suggests that pathogenic microbes can still grow on a medium although the growth rate is different. Microbial pathogens can still grow because pathogenic microbes still use the existing glucose to grow. The growth pattern of Total Bacteria, Lactobacillus, Bifidobacteria, Bacteroides, and Clostridium in Gembolo oligosaccharides extract can be seen in Figure 1.

Figure 1. The growth pattern of Total Bacteria, Lactobacillus, Bifidobacteria, Bacteroides, and Clostridium in gembolo oligosaccharides extract

The growth of all colon bacterium genus will be used to calculate the value of the prebiotic index. The prebiotic index values describe the prebiotic properties of oligosaccharides that can spur the growth of probiotic bacteria compared with pathogenic microbes [22]. The oligosaccharide index of the gembolo flour is determined based on microbial growth from 0 hour to 48th hours incubated at 37 °C under anaerobic conditions. The value of the prebiotic index can be seen in Table 3.

Table 3. Prebiotic Index of Gembolo Flour Oligosaccharide Extract

| Time (hours) | Prebiotic Index |
|--------------|----------------|
| 24           | 0.3876         |
| 48           | 1.2045         |

As shown in Table 3, the value of the prebiotic index at the 24th hour was 0.3876, and at the 48th hour, it increased to 1.2045. The increase in prebiotic index values indicates that Gembolo flour oligosaccharide extract is able to support the growth of colonic microbes (Lactobacillus and Bifidobacteria) well, although the growth of pathogenic microbes (Bacteroides and Clostridium) also increases. An increase in the number of pathogenic microbes can be caused by pathogenic microbes still using glucose present in the media to perform their metabolism, thus increasing their growth. Prebiotic index values obtained are quite low when compared with other prebiotic index values of oligosaccharides such as inulin which has a prebiotic index of 1.82 and lactulose which has a prebiotic index of 4.90 [13]. Oligosaccharides present in the extract are expected to inhibit the growth of pathogenic microbes such as Clostridium and Bacteroides, but the extract also contains high monosaccharide that can be used the microbial pathogens to metabolism and growth. In addition, the pH of the media is still not able to inhibit the growth of pathogenic microbes because the pH is still not low enough so that pathogenic microbes can still grow.
4. Conclusion
Oligosaccharides profile in gembolo flour extract were 0.76% Inulin; 0.07% Raffinose; and 0.09% Lactulose w/v. The highest oligosaccharide content in gembolo extract was inulin, and the largest monosaccharide content was fructose. Gembolo flour oligosaccharides extract has prebiotic potency related to the value of the prebiotic index at 48 hours which about 1.2045.

5. References
[1] Hasler C M 1998 “Functional Foods: Their Role in Disease in: Developing New Food Products for a Changing Prevention and Health Promotion” Food Technology 52 2 57-62
[2] Supriya A, Yadav, Snehal S G, Vikram B L, Smita S N and Vaishali V A 2014 In vitro screening of indigenous plant materials for prebiotic potential Int. J. Curr. Microbiol. App. Sci. 3 11 137-150
[3] Miyazato S, Nakagawa C, Kishimoto Y, Tagami H, Hara H 2010 Promotive effects of resistant maltodextrin on apparent absorption of calcium, magnesium, iron and zinc in rats Eur. J. Clin. Nutr. 49 165-171
[4] Roberfroid M B 2001 Prebiotics: preferential substrates for specific germs? Am. J. Clin. Nutr. 73 406-409
[5] Ghosh S, More P, Derle A, Patil A B, Markad P, Asok A 2014 Diosgenin from Dioscorea bulbifera: Novel Hit for Treatment of Type II Diabetes Mellitus with Inhibitory Activity against α-Amylase and α-Glucosidase PLoS ONE 9 9 e106039
[6] Ghosh S, More P, Nitnavare R, Jagtap S, Chippalkatti R, Derle A, Kitture R, Asok A, Kale S, Singh S, Shaikh M, Ramanamurthy B, Bellare J, Chopade 2015 Antidiabetic and Antioxidant Properties of Copper Nanoparticles Synthesized by Medicinal Plant Dioscorea bulbifera Journal of Nanomedicine & Nanotechnology S6 007
[7] Muchtadi, D 1989 Laboratorium Procedure of Nutrient Evaluation (Bogor: PAU Pangan dan Gizi IPB)
[8] AOAC (Association of Official Analytical Chemist) 2005 Official Methods of Analysis of the Association of Official Analytical Chemists (New York : Chemist Inc.)
[9] Apriyantono A, Fardiaz D, Puspitasari N, Yasni S and Budiyanto 1989 Laboratorium Procedure of Food Analysis (Bogor : IPB Press)
[10] Vulevic J, Rastall R A, Gibson G R 2004 Developing a quantitative approach for determining the in vitro prebiotic potential of dietary oligosaccharides FEMS Microbiol Lett 236 153–159
[11] Vardakou M, Palop C N, Christakopoulou P S, Faulds C B, Gasson M A, Nabad A 2008 Evaluation of the prebiotic properties of wheat arabinoxylan fraction and induction of hydrolase activity in gut microflora International Journal Food Microbiology 123 166-170.
[12] Polnaya, F J 2013 Physisciochemistry Characteristic and Prebiotic Potency of Phosphate Sago Starch (Metroxylon rumphii) Dissertation Faculty of Agricultural Technology Universitas Gadjah Mada
[13] Palframan R, Gibson G R, Rastall R A 2003 Development of a quantitative tool for the comparison of the prebiotics effect of dietary oligosaccharides LettnAppl Microbial 37 281-284
[14] Niness KR 1999 Inulin and oligofructose: what are they? J. Nut. 129 1402S–6S
[15] Kalyani N K, Kharb S, Thompkinson D K 2010 Inulin Dietary Fiber with Functional and Health Attributes— A Review Food Rev. Intern. 26 189–203
[16] Wilson B, Whelan K 2017 Prebiotic inulin type fructans and galacto oligosaccharides: definition, specificity, function, and application in gastrointestinal disorders Journal of Gastroenterology and Hepatology 32 64-68
[17] Rubel I, Perez E, Genovesi D, Manrique G 2014 In vitro prebiotic activity of inulin-rich carbohydrates extracted from Jerusalem artichoke (Helianthus tuberosus L.) tubers at different storage times by Lactobacillus paracasei Food Research International 62 59-65
[18] Caleffi E, Krausova G, Hyrslova I, Paredes L, Santos M, Sassaki G, Goncalves R, Oliveira A 2015 Isolation and prebiotic activity of inulin-type fructan extracted from *Pfaffia glomerata* (Spreng) Pedersen roots *International Journal of Biological Macromolecules* **80** 392-399.

[19] Samal L, Chaturvedi V B, Pattanaik A K 2017 Effects of dietary supplementation with Jerusalem artichoke (*Helianthus tuberosus* L.) tubers on growth performance, nutrient digestibility as well as activity and composition of large intestinal microbiota in rats *J. Anim. Feed Sci.* **26** (1) 50–58

[20] Miyaguchi Y, Tomatsuri T, Toyoda A, Inoue E, Ogawa Y 2015 Effect of Yacon Tuber (*Smallanthus sonchifolius*)-derived Fructooligosaccharides on the Intestinal Flora and Immune System of OVA-sensitized BALB/c Mice *Food Science and Technology Research* **21** 2

[21] Mishra C & Lambert J 1996 Production of antimicrobial substances by probiotics *Asia Pac J Clin Nutr* **5** 20–24

[22] Mandalari G, Nueno-Palop C, Bisignano G 2008 Potential prebiotic properties of almond (*Amygdalus communis* L.) seeds *Appl Environ Microbiol* **74** 4264–4270