Inulin Extraction and Characterisation of Fresh and Chip Gembili (*Dioscorea Esculenta*) Extract by Ultrasound-assisted Extraction

A Hilman[1]*, E. Harmayani[2], M. N. Cahyanto[2]
[1]*Postgraduate Program of Food Science and Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Sleman 55281, Yogyakarta, Indonesia*
[2]*Department of Food and Agricultural Products Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Sleman 55281, Yogyakarta, Indonesia*

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Abstract: Inulin has the prebiotic component to improve health and reduce the risk of digestive tract disorders. Gembili (*Dioscorea esculenta*) is one of inulin source found in Indonesia. Fresh Gembili has a relatively short shelf-life so it needs to be drying into chips. Inulin extraction was the factor that can affect the characteristics of gembili extract. The aim of this research was to determine the effect of ultrasound-assisted extraction on the inulin extraction stage to the yield and the characteristics of fresh and chip gembili extract. The study was conducted in two stages: (1) inulin extraction from fresh and chip gembili with ultrasound-assisted extraction and compared by non-ultrasound extraction; (2) characterisation of physical and chemical properties. The results showed that inulin extraction by ultrasound-assisted extraction was not different significantly to the yield (30.78%-32.47%); degree of white (92.18-93.69); pH (6.55) and solubility at 25^{O}C (11.26%-12.75%), 60^{O}C (22.50%-25.97%), 90^{O}C (36.34%-37.71%) compared by non-ultrasound extraction. Gembili extract from fresh and chip gembili by ultrasound-assisted extraction have inulin content was about 10.00%-21.13%; inulin purity was about 61.57-119.22 mg/kg; and viscosity becomes smaller as the temperature increases. It could be concluded that inulin can be extracted from fresh and chip gembili.

1 INTRODUCTION

Inulin is a prebiotic component consisting of a D-Fructose unit monomer connected by a β-(2→1) bond and has a D-Glucose terminal group connected to an α-(1→2) bond. The arrangement of fructose monomers makes inulin cannot be hydrolyzed in the digestive system. However, inulin can be fermented by microbiota in the digestive tract in the colon by *Bifidobacterium* and *Lactobacillus* (Li et al., 2015). The results of inulin fermentation in the digestive system are short-chain fatty acids that can be used by cells to stimulate the growth of intestinal mucosal cells and become the main source of cell energy (Pompei et al., 2008). In addition, the results of inulin fermentation have immunomodulatory effects and increase the mineral absorption (Dominguez et al., 2014; Panchev et al., 2011). The food industry has been using inulin in various applications in various products today.

This sparked a great deal of research and publication about the production of inulin from different types of plants (Roberfroid, 2005). Inulin has been commercially produced from chicory root (*Chicorium intybus*) and Jerusalem artichoke tubers (*Helianthus tuberosus*) in some western countries such as America, England and some European countries.

According to the research that has investigated by Winarti et al. (2011) and also Zubaidah and Akhadiana (2013) reported that one of the major sources of inulin in Indonesia can be found in plants such as gembili (*Dioscorea esculenta*) tuber. Winarti et al. (2011) reported that the content of inulin in fresh gembili was 14.77% (db). While Ciptaningrum (2015) reported that the inulin extraction method of gembili chip with water addition ratio resulted in a yield of 36.40% (db) with an inulin content of 21.64%.

According to previous study, gembili has the potential to serve as a raw material on inulin extraction. However, gembili has a relatively long

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harvest time of about seven to nine months. In addition, the gembili that is kept in fresh condition only has a relatively short shelf-life of about 10 to 14 days in room temperature (Kasno, Saleh and Ginting, 2006). These problems can be solved by giving preliminary treatment at fresh gembili processed into the chip. Only a few studies have studied the extraction of inulin from the materials of the chip, among others, from the Jerusalem artichoke chip (Saengthongpinit, 2005; Bekers et al., 2008) and *Cichorium intybus L* dried (Park, de Oliveira, Brod, 2006). Current research has studied the extraction of inulin from raw materials in the form of gembili chip (Ciptaningrum, 2015).

Arumdinari (2015) reported that the inulin extraction method performed was the development of the method Gupta, Kaur and Kaur (2003) which used the principle of extraction by heating in boiling water (90^{o}C) for 20 min, filtrate freezing process for 24 h, inulin precipitation with ethanol 20% and drying used by cabinet drying in overnight at 50^{o}C. The study obtained in inulin yield of 43.39% (db) and inulin content of 28.89%.

Therefore, research is needed to improve the inulin yield of the gembili on an industrial scale. The ultrasound-assisted extraction is used as a new method capable of extracting inulin from various plant tissues. The aim of this study was to determine the effect of ultrasound-assisted extraction on the stage of inulin extraction to the yield and the characteristics of fresh and chip gembili extract.

2 MATERIALS AND METHOD

The research was conducted in two stages: 1) inulin extraction from fresh and chip gembili by ultrasound-assisted extraction and compared by non-ultrasound extraction and 2) characterization of physical and chemical properties.

2.1 Materials

The research material was harvested from fresh gembili tubers obtained from Watubonang Village, Tawangsari District, Sukoharjo Regency, Central Java Province. Gembili chip was a fresh gembili that has been sliced thin chip-shaped and dried at 50^{o}C in cabinet drying for 12 hours. Another materials used are inulin (C6H10O5)n standard produced by Sigma Aldrich and fructooligosaccharides for inulin purity analysis using HPLC (High Liquid Performance Chromatography).

2.2 Instruments

The research instruments used were waterbath shaker, HPLC Shimadzu, chromameter CR-400 (*Konica Minolta*), viscosity Brookfield LVDV-II+P, column ion-exclusion Aminex HPX-87H 150X7.8 mm ID (*BioRad, Watford, Herts*), freeze drying, centrifuge, sonicator bath.

2.3 Statistical Analysis

The research method was conducted by Complete Randomize Experimental Design. The treatment factor was the type of material consist of 1) fresh gembili with non-ultrasound extraction (G1) and by ultrasound-assisted extraction (G2); 2) chip gembili with non-ultrasound extraction (G3) and by ultrasound-assisted extraction (G4). Each treatment was repeated 3 times to minimize experimental error. The analysis was performed by analysis of variance (ANOVA) and followed by Duncan's test with a significance level of 5%. Statistical data is calculated by SPSS 17.0 software.

2.4 Inulin Extraction

Inulin extraction from fresh and chips gembili in this study used the method by Arumdinari (2015) with modification. Fresh gembili cleaned, peeled and mixed in 800 ml of hot water (80^{o}C). Gembili crushed with blender for 3 min to get the puree. The puree was extracted used ultrasound-assisted in a sonicator bath for 5 min. As a comparison, the other puree was extracted used the treatment non-ultrasound extraction. Both of the puree were extracted used hot water extraction on waterbath shaker at 80^{o}C for 60 min at 80 rpm. The hot puree was filtered with four layers of cotton to get the filtrate. The filtrate was added to 1000 ml of hot water (80^{o}C) and reheated by the same method. In the third stage of heating, the filtrate was added 500 ml of ethanol 96% and reheated on a waterbath shaker at 80°C for 60 min at 80 rpm. The filtrate was then frozen at -20°C for 24 h. The frozen filtrate was thawed for 12 h and centrifuged at 1500 rpm for 15 min to obtain the natan. The supernatant section is separated from natan as it contains the impurities component. White natan is taken and washed with ethanol 20% and centrifuged again (Kaur and Gupta, 2015). Furthermore, natan was washed by used aquades as a neutralizer. Natan was dried in freeze drying at -87^{o}C for 48 h. (The same experiment was also done for the material of gembili chip).

2.5 Calculation of the Yield

The yield is the ratio between the weight of the dried gembili extract (a) and the weight of the gembili material (b). The yield can be used to determine the depreciation or addition of weight after processing.

2.6 Analysis of Moisture Content

The sample of 1-2 g was inserted into an aluminum foil plate that has been dried in an oven at 105°C. It was known to be constant to weight (a). The sample dried at 105°C for 3-5 h and cooled in a desiccator, then weighed. Heated again in the oven for 30 min, chilled and weighed. This treatment was repeated until it reaches a constant (b).

$$\frac{a-b}{a} \times 100\% \quad (1)$$

2.7 Analysis of Inulin Content (Li et al., 2015)

Inulin content can be calculated by differences between the difference in total sugar content used by phenol-sulfuric acid and inulin as standard (Dubois et al., 1956) with reducing sugar content used by the method of 3,5-dinitrosalicylic acid (DNS) and D-fructose as standard (Miller, 1959).

2.8 Analysis of Solubility (Jiang et al., 2013)

Solubility assay was performed by heating 50 ml inulin solution 1% w/v (S). The solution put into the water at 25°C, 60°C and 90°C and stirred for 15 min. The solubility time was calculated used the stopwatch until all dissolves. The solution filtered by a filter paper that has been known the weight (K1). The solution left in the filter paper dried at 105°C for 3 h and weighed (K2).

$$\frac{(S \times TSS) - (K2 - K1)}{S \times TSS} \times 100\% \quad (2)$$

2.9 Analysis of Viscosity

Prepared fresh and chip of gembili extract solution of 10% with aquades and heated while stirring to a temperature at 100°C. The viscosity was measured used by Brookfield LVDV-II+P viscosimeter. Spindles were used adjusted to the viscosity of solution. The analysis began with temperatures of 90°C, 80°C, 70°C, 60°C, 50°C, 40°C and 30°C. The results were compared with inulin standard solution.

2.10 Analysis of White Degree (Takeuchi and Nagashima, 2011)

Coordinate of L* a* b* is measured by chromameter CR-400 (*Konica Minolta*) with visual angle 20. The color parameters were expressed as follows: brightness (L*), redness (a*) and yellowish (b*). The lowest value of L* was 0 which indicate by blackness and the highest was 100 indicate by white. a* negative value indicate by green and positive showed by red. While the negative b* value showed by blue and positive colors indicate by yellow.

$$100 - \sqrt{\left((100 - L)^2 + (a^2 + b^2)\right)} \quad (3)$$

2.11 Analysis of Inulin Purity (Retnaningtyas, 2012)

The inulin purity test was measured used by HPLC with Aminex Ion-Exclusion HPX-87C (250mm x 4mm) column, 410 model water refractive detector and LCHE Waters model M-45 pump. Aquades water were used as a mobile phase with a speed of 0.5 ml/min, injection volume 20 µl. The column temperature was set at 60°C and detector at 40°C. The inulin standard was used by inulin (C6H10O5)n obtained from Sigma Aldrich.

2.12 Measurement of pH

The pH measurement was performed by dissolving the sample with a concentration of 10% (w/v). The pH measurement analysis aims to determine the condition of substrate acidity in the dissolved sample. The pH Measurement was made in triplicate. The pH measurement was performed used by pH-meters.

3 RESULTS AND DISCUSSION

3.1 Yield

The average yield of fresh and chip gembili extract by ultrasound-assisted extraction were 32.47% and 30.78% respectively. The ultrasound-assisted extraction was not different significantly on the yield of fresh and chip gembili extract. However, the yield of fresh and chip gembili extract obtained increased

through the extraction process used by ultrasound-assisted extraction (Table 1). Lingyun et al. (2007) reported that the efficiency of inulin extraction will increase efficiency by ultrasound-assisted extraction. ultrasound extraction with the cleaning bath is non-destructive to the sample which will eliminate the possible contamination and loss of the extract. Moreover, the cleaning bath is usually much quieter than the probe horn during the operation. Therefore, an ultrasonic cleaning bath might be more convenient and efficient for the inulin extraction.

Table 1: Yield, inulin content, solubility at 25ºC, inulin purity and degree of white from fresh and chip of gembili extract by non-ultrasound and ultrasound-assisted extraction.

Characteristic of physico-chemistry	Fresh gembili extract	
	non-ultrasound	ultrasound
Yield (%)	26.91 ± 5.56^{ab}	32.47 ± 0.61^{b}
Inulin content (%)	6.35 ± 0.13^{a}	0.00 ± 0.08^{c}
Solubility 25ºC (%)	11.26 ± 0.03^{a}	11.48 ± 0.09^{a}
White degree	92.37 ± 0.32^{a}	2.44 ± 0.30^{a}
Inulin purity (mg/kg)	56.55 ± 19.80	61.57 ± 0.56
Characteristic of physico-chemistry	Chip gembili extract	
	non-ultrasound	ultrasound
Yield (%)	25.23 ± 1.18^{a}	30.78 ±4.21^{ab}
Inulin content (%)	7.67 ± 0.14^{b}	1.13 ± 0.18^{d}
Solubility 25ºC (%)	12.41 ± 0.02^{b}	12.75 ± 0.32^{b}
White degree	92.18 ± 0.49^{a}	2.69 ± 0.20^{a}
Inulin purity (mg/kg)	96.70 ± 5.70	119.22 ±0.74

Description: different superscripts on the same line showed significant differences ($p < 0.05$)

3.2 Moisture Content

The moisture content of fresh and chip gembili extract by ultrasound-assisted estraction were 11.48% and 11.28% respectively. Winarti et al. (2011) reported that inulin extracted from fresh gembili tubers dried by cabinet drying method with temperature at 60ºC had a moisture content of up to 13.5%. While Franck (2007) reported that the percentage of inulin standard from chicory had dry material of 95%, which means that inulin standard from chicory only had moisture content of 5%. Inulin moisture content of information should be known to limit the amount of water in the material that will affect the resistance to damage caused by microorganisms.

3.3 Inulin Content

Fresh and chip gembili extract by ultrasound-assisted extraction have inulin levels of 10.00% and 21.13% respectively. Ciptaningrum (2015) reported that the inulin content of gembili chip ranged between 21.13% to 21.64%. The ultrasound-assisted extraction has provided an increase in inulin content of fresh and chip gembili extract (Table 1). Ultrasound-assisted extraction released of inulin compounds within the plant cell vacuoles and diffuses out of the cell with kinetic waves derived from the resulting vibration (Vinatoru, 2001). Analysis of inulin content by the spectrophotometric method still reads all the sugars present in the material. Thus the readable inulin content may be a component other than inulin, such as starch, soluble fiber and other carbohydrates. Therefore, it is necessary to continue a more accurate analysis of inulin levels by using methods other than spectrophotometry, such as HPLC.

3.4 Solubility

The quality of inulin can be measured by the level of solubility in water. This parameter is one of the physical properties possessed by inulin. Fresh and chip gembili extract by ultrasound-assisted extraction have a solubility at 25ºC by 11.48% and 12.75% respectively. Franck (2007) reported that the rate of inulin solubility at 25°C was 12% (w/v). However, the solubility of inulin will increase as the temperature increases as well. The statistical results show that there is increased solubility of the gembili extract between the fresh and chip, where the solubility of the chip gembili extract is higher than that of fresh gembili extract. Lee and Cheng (2006) reported that drying techniques using hot temperatures can lead to smaller particle sizes that exhibit better redispersing properties.

3.5 Viscosity

The viscosity is measured at a concentration of 10% (w/v) and starts at 90°C until the temperature drops at 30°C. The viscosity of fresh and chip gembili extract by ultrasound-assisted extraction can be seen in Figure 1. The results showed that the higher

temperature so the viscosity value will become smaller. The temperature factor becomes the determinant of the small value of the inulin viscosity as the distance between the molecules further and the frictional force decreases. Wada et al. (2005) reported that the viscosity value may increase due to the increase in molecular weight and temperature drop. Bouchard, Hofland and Witkamp (2007) reported that the value of inulin viscosity at 37°C with a 10% concentration of 1.12 mpa-s. The low level of inulin viscosity is a characteristic of standard inulin physical properties commonly used in food products (Franck, 2007).

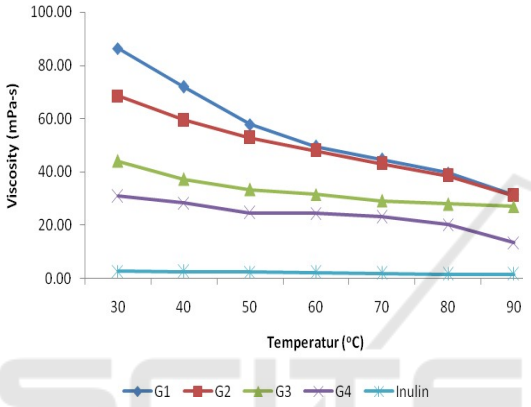


Figure 1: The viscosity of gembili extract from fresh gembili by non-ultrasound (G1) and ultrasound (G2), chip gembili by non-ultrasound (G3) and ultrasound (G4), and inulin.

3.6 White Degree

Fresh and chip gembili extract by ultrasound-assisted extraction have a white degree of 92.44 and 92.69 respectively. The results showed that the ultrasound-assisted extraction did not affect a white degree of fresh and chip gembili extract. The gembili extract can be seen in Figure 2. Color is one of the standard physical properties of inulin. Visual appearance is a determinant of inulin quality. Franck (2007) reported that the appearance of chicory and arthicoke colors is white. However, the white degree obtained was better than previous studies using cabinet drying with a white degree of 81.39 (Ciptaningrum, 2015).

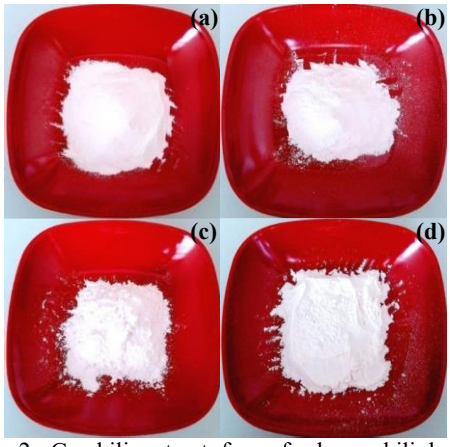
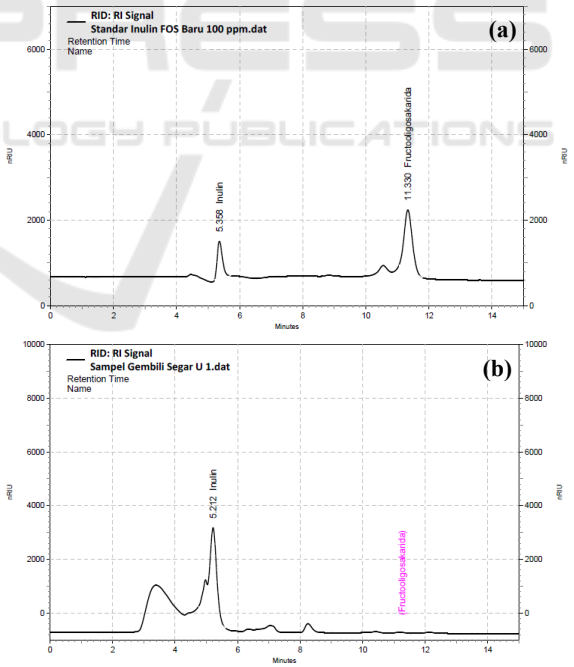


Figure 2: Gembili extract from fresh gembili by non-ultrasound (a) and ultrasound (b), chip gembili by non-ultrasound (c) and ultrasound (d).

3.7 Inulin Purity

Figure 3. point (a) showed that there were two main peaks used as peaks of inulin and FOS standard with a retention time of 5.3 and 11.3 min respectively. Figure 3. points of (b) and (c) were the result of chromatograms of gembili extract that have the same retention times as inulin and FOS standard.



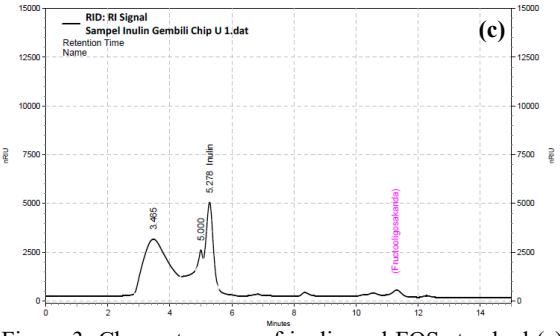


Figure 3: Chromatograms of inulin and FOS standard (a), fresh gembili extract by ultrasound (b) and chip gembili extract by ultrasound (c).

However, since the concentrations of FOS standard still exist that have not been detected in some chromatograms then the data is not shown. The inulin purity is calculated by the multiplication of concentration obtained by the diluting factor of the gembili extract and divided by the initial weight of the gembili extract. The inulin purity from gembili extract is presented in Table 2.

Table 2: Inulin purity of gembili extract

Gembili extract		ulin purity (mg/kg)
Fresh	Non-ultrasound	56.55 ± 19.80
	Ultrasound	61.57 ± 0.56
Chip	Non-ultrasound	96.70 ± 5.70
	Ultrasound	119.22 ± 0.74

Data as mean values ± standard deviation (n = 2)

Table 2. showed that there is an increase in inulin purity of fresh and chip gembili extract by ultrasound-assisted extraction. Fresh and chip gembili extract by ultrasound-assisted extraction have an inulin purity of 61.57 mg/kg and 119.22 mg/kg respectively. Zubaidah and Akhadiana (2013) reported that inulin concentration in the fresh gembili extract of 67.66 mg/kg.

This suggests that ultrasound-assisted extraction of inulin extraction from fresh and chip gembili can improve inulin purity. Lingyun et al. (2007) reported that extraction by ultrasound-assisted extraction effective can cause disruption of plant cell walls to break and release compounds extracted from within the cell wall.

3.8 pH

The pH parameter becomes important because inulin is an additive in various food products to increase functional value. So the base-acid information from gembili extract can be used as a basic knowledge in its use in various food products. The average pH value of gembili extract from fresh gembili non-ultrasound, fresh gembili ultrasound, chip gembili non-ultrasound, chip gembili ultrasound extract were 6,55. The pH value of 6.55 is still within the normal pH range. Franck (2007) reported that the standard inulin pH range of 10% (w/v) concentration was 5-7. So this can make the gembili extract as fortification or food additives into food products.

4 CONCLUSIONS

The inulin extraction of fresh and chip gembili by ultrasound-assisted extraction was not different significantly to the yield (30.78%-32.47%); degree of white (92.18-93.69); pH (6.55) and solubility at 25ºC (11.26%-12.75%), 60ºC (22.50%-25.97%), 90ºC (36.34%-37.71%) compared by non-ultrasound extraction. Gembili extract from fresh and chip gembili by ultrasound-assisted extraction have inulin content was about 10.00%-21.13%; inulin purity was about 61.57-119,22 mg/kg; and the viscosity becomes smaller as the temperature increases. It could be concluded that inulin can be extracted from fresh and chip gembili. Study of inulin extraction from fresh and chip gembili by ultrasound-assisted extraction needed to be developed by adding a longer time variation in the future.

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