

Characterization of Chemical Properties of Inulin Isolated from Yacón Tuber

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ABSTRACT

Inulin is a polysaccharide with linear fructan chain structure. Food industries widely use inulin as a low calorie sugar substitutions. Yacón (*Smallanthus sonchifolius*) tuber that are easily grown and contain higher inulin can be explored as local inulin sources. This research aimed to characterize the chemical properties of inulin isolated from yacón tuber. The methods of this study include proximate analysis of yacón tuber, inulin isolation, and characterization of purified inulin using FTIR. The results showed that yacón consisted of 91.23 water (analyzed as moisture), 0.12 proteins, 0.58 fats, 0.52 crude fibers, and 7.34% carbohydrates. The yield of inulin extracted from yacón tuber was 4.86% whereas its purity and actual content of inulin were 44.23 and 2.15%, respectively. The Osazon test revealed that the isolated inulin has similar crystalline with that from chicory. Based on characterization using the FTIR spectrophotometer, the isolated inulin had functional groups of C-O, C-H, CH₂, O-H, and C=O. The spectrum of the isolated inulin has similarities with chicory inulin. Therefore, yacón tuber could be considered as a potential local inulin source in Indonesia.

Keywords: chemical characterization, inulin, prebiotics, tuber, yacón

INTRODUCTION

Dietary prebiotics is defined as selectively fermented ingredients that result in specific changes in the composition or activity of the gastrointestinal microbiota, thus conferring benefits upon host health (Valcheva and Dieleman, 2016). Several lactic acid bacteria that can use inulin as a carbon source are *Lactobacillus rhamnosus* and *Pediococcus pentosaceus* (Nuraida *et al.*, 2011). Prebiotic from daluga (*Cyrtosperma merkusii* (Hassk.) Schott) flour can stimulate bacterial growth such as *L. plantanarum* (Purnamasari *et al.*, 2019). The demand for prebiotics is increasing rapidly. The world prebiotics market showed more than 400 prebiotic food products from more than 20 companies, some of them use oligosaccharides as prebiotics. The global market of functional foods is estimated up to US\$ 33 billion (FAO, 2018). Indonesian inulin imports in 2005 are 160.7 million and grew double in 2009 for up to USD 306.5 million.

Inulin is one of the widely used prebiotic in the world. It is a fructan polymer which consists of fructose subunits with β -2,1 glycosidic bond and polymerization degree from 2 to 60 (Mensink *et al.*, 2015). Inulin is common to be found in the food industry as sugar substitution, stabilizing agent, texture changing agent, and functional food development (Shoab *et al.*, 2016). Inulin can be used as a sugar substitution with low calories. Faradila *et al.* (2016) showed that

inulin also plays a role in the health of *pelung*-leghorn chicken by optimizing microflora in the intestine.

World inulin sources are supplied by extraction from Jerusalem artichoke (*Helianthus tuberosus*) and chicory (*Chicoryum intybus* L) plantation, but those plants distribution is limited and nowhere to be found in Indonesia due to climate reasons (De Mastro *et al.*, 2004). Exploration of the inulin producer plant has been done in Indonesia and showed that *Dahlia sp.* L., *Dioscorea esculenta*, and *Pachyrhizus erosus* tuber. However, insulin concentration in those plants is very small and cultivation of the plants also challenging as well (Zubaidah and Akhadiana, 2013). These problems drive the need to find an alternative plant for inulin production in Indonesia.

Yacón (*Smallanthus sonchifolius*) is a wild plant from the Asteraceae family common to be found in a riverbank or as hedgerows. It can live in various types of soil and pH range with good drainage. Indonesian people cultivate yacón mainly on Wonosobo region, Central Java Province to take the leave to make herbal medicine for treating diabetes. Each plant can produce 10 kg tuber, with the weight of each tuber up to 500 g (Pahlawan and Oktaria 2016).

Yacón tuber contains oligosaccharides with 2→1 glycosidic bond refer to inulin. Sousa *et al.* (2015) proved that yacón tuber powder exhibited prebiotic activity *in vitro*. Thus, yacón isolated inulin has the potential to be used as a source of inulin production in Indonesia. This research aimed to characterize chemical properties of inulin from yacón tuber

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originated in Indonesia. Additionally, the content of isolated inulin from yacón tuber was measured, and also compared with previously reported known tubers.

MATERIALS AND METHODS

Materials

The main material in this research was yacón tubers collected from Yakon Natura Farm, Kertek Regency, Wonosobo Town, Central Java Province, Indonesia.

Chemical properties

Chemical properties of inulin were measured based on AOAC (2015) method, include moisture, ash, fat, crude fiber, and carbohydrate contents. Protein content was measured using Bradford's reaction and bovine serum albumin was used as standard.

Measurement of water content

Porcelain plates were heated at 105°C oven (Memmert, Schwabach, Germany), then cooled in a desiccator and weighed. Then 2 g of sample were put in the porcelain plates and heated at 105°C for an hour in an oven. After that, the porcelain plates were put into the desiccator immediately and weighed after reaching room temperature. Heating was repeated until a permanent weight was obtained. The remaining samples were counted as total solids and lost weight as moisture content.

Moisture Content (%) =

$$\frac{\text{initial weight} - \text{final weight (g)}}{\text{initial weight (g)}} \times 100\% \dots \dots \dots (1)$$

Measurement of ash content

Ash content was determined by weighing the remaining minerals as a result of the combustion of organic material at temperatures around 550°C furnace. The porcelain dishes were heated, cooled in a desiccator and weighed when the room temperature is reached. The sample of 2 g was put into the porcelain dish, then weighed in a cup and then burned at 550°C furnace until the ash was gray or of constant weight. Subsequently, porcelain dishes were cooled in a desiccator and weighed as soon as it reached room temperature.

$$\text{Ash Content (\%)} = \frac{\text{ash weight (g)}}{\text{sample weight (g)}} \times 100\% \dots \dots (2)$$

Measurement of fat content

A sample of 3 g was put into Whatman filter paper grade 597 made like a bag. Then put into the

Soxhlet apparatus and extracted for 6 h using petroleum benzene (Merck, Darmstadt, Germany). Previously the round bottom flask and boiling stone were dried in a 105°C oven for an hour, cooled in a desiccator and weighed. After sufficient extraction, the solvent in the round bottom flask was evaporated until it is finished and then cooled in a desiccator and weighed until a constant weight is obtained.

Fat Content (%) =

$$\frac{\text{Weight of extracted fat (g)}}{\text{Weight of sample (g)}} \times 100\% \dots \dots \dots (3)$$

Measurement of crude fiber

The samples of 3 g were extracted by the Soxhlet method in hexane (Merck, Darmstadt, Germany) 3 times. The sample was dried and dissolved with 50 mL of 1.25% H₂SO₄ (Smartlab, Tangerang, Indonesia) solution, then boiled for 30 min in a water bath (Memmert, Schwabach, Germany). Next, 50 mL of 3.25% NaOH (Ajax Finechem, Seven Hills, Australia) was added and boiled again for 30 min. The sample was filtered using a Buchner funnel containing Whatman 41 filter paper which has been dried in the hot condition and weighed. The deposits contained in filter paper were successively washed using 1.25% H₂SO₄, hot water, and 96% ethanol (Smartlab, Tangerang, Indonesia). The filter paper and its content were removed and dried in an oven at 105°C, cooled, and weighed.

Crude Fiber Level (%) =

$$\frac{\text{crude fiber weighed (g)}}{\text{sample weighed (g)}} \times 100\% \dots \dots \dots (4)$$

Measurement of protein levels

Protein standard curves were determined using standard protein solutions, Bovine Serum Albumin (BSA) (Abcam, Eugene, USA) with concentrations of 0, 40, 80, 120, 160, and 200 µg/mL, the volume was 100 µL. Then each tube was added with 5 mL Bradford reagent (Abcam, Eugene, USA). The tubes were covered with aluminum foil and shaken. After that, tubes were incubated at room temperature for 5 min. The absorbance was obtained using a spectrophotometer (Genesys Thermo Scientific, Houston, USA) at a wavelength of 615 nm three times. Furthermore, the protein content of the sample was calculated based on the standard protein curve obtained.

Measurement of carbohydrate content

Carbohydrate content obtained using the difference method. All proximate tests on a wet basis are added up and then added to the carbohydrate content until the results reach 100%.

Inulin isolation

The inulin isolation process was performed by following the method of Petkova *et al.* (2018b) with modification. Yacón tubers were washed, peeled, and cut into small pieces. Then tubers were mixed with 2 times the volume of water and homogenized using a juicer. NaOH (Ajax Finechem, Seven Hills, Australia) was added into the solution until the pH value raised 7 and heated at 80°C in a water bath (Mettler, Schwabach, Germany) for 30 min. The solution was filtered, then the filtrate was mixed with ethanol 30% (Smartlab, Tangerang, Indonesia), the volume was 40% of the filtrate. Then the solution was stored at room temperature for 24 h. The sediment that contains inulin was taken out. Then the wet inulin (sediment) was added with water and refined. Subsequently, the inulin was dried at 50°C in the oven (Mettler, Schwabach, Germany) for 3 h and mashed up to become inulin powder.

Purity measurement of isolated inulin

The purity of inulin was determined based on the difference between the amount of inulin reducing sugar before and after hydrolysis then multiplied by a constant (0.995) according to the research of Saengkanuk *et al.* (2011). Reducing sugar measurements were carried out using the dinitro salicylate (DNS) method. The DNS method was carried out by mixing 1 mL of the sample with 3 mL of DNS reagents (Sigma Aldrich, Darmstadt, Germany). Then the solution was soaked in boiling water for 5 min and let it cool down at room temperature. The solution was then analyzed using a spectrophotometer (Genesys Thermo Scientific, Houston, USA) at 550 nm wavelength. Standard curves were made using glucose (Ajax Finechem, Seven Hills, Australia) solutions in the range of 0-2 mg/mL. Inulin was hydrolyzed in 0.2 M HCl (Smartlab, Tangerang, Indonesia) with a ratio of 1:25, then placed in a 100°C water bath (Mettler, Schwabach, Germany) for 45 min. The solution was allowed to cool down, then added with NaOH (Ajax Finechem, Seven Hills, Australia) until it reaches pH 7, then the solution was measured its reducing sugar content.

FTIR analysis of isolated inulin

Functional group analysis was conducted using the Osazon method (Babu *et al.*, 2015). Two milliliters of test solution; isolated inulin, inulin from chicory (Sigma Aldrich, Germany), glucose (Ajax Finechem, Seven Hills, Australia), fructose (Ajax Finechem, Seven Hills, Australia), or starch (Ajax Finechem, Seven Hills, Australia) were mixed with 10 mg of phenylhydrazine (Merck, Darmstadt, Germany) and sodium acetate (Ajax Finechem, Seven Hills, Australia). Then the mixture was heated for 1 min and let it cool. Then one drop of the mixture was observed

under a light microscope (Olympus, Tokyo, Japan) 40 times magnification.

Analysis of functional groups was conducted using FTIR spectrophotometers (Melanie *et al.*, 2015). The isolated inulin powder was mixed with KBr (ABB, Zurich, Switzerland) at a ratio of 300: 1, then compacted using a hydraulic pressure device (ABB, Zurich, Switzerland) into a pellet. Furthermore, the pellet was placed in a pellet holder and placed between the light sources of the FTIR ABB 3000 spectrophotometer (ABB, Zurich, Switzerland). The software used was the Horizon MB built in the ABB 3000 FTIR instrument which has been validated by a measurement before measuring the sample. Sample measurements were carried out using a resolution of 16 cm⁻¹ with a wavelength range of 4000-400 cm⁻¹ and the spectrum of the yacón inulin spectrum was obtained. The same procedure was also used to find out the spectrum results from inulin from chicory. Furthermore, the spectrum of yacón inulin was aligned with the chicory inulin standard based on the wavenumber.

RESULTS AND DISCUSSION

Chemical characteristics of yacón tuber

Table 1 shows the moisture content, ash content, crude fiber content, fat content, protein content and carbohydrate content of yacón tuber were 91.23, 0.21, 0.52, 0.58, 0.12, and 7.34% wet weight or wet basis (wb), respectively. The chemical characteristics of the yacón tuber were compared with the dahlia tuber as a local inulin producer and chicory tuber as a producer of the world commercial inulin (Table 1). The dahlia tuber has moisture content, ash content, crude fiber content, fat content, protein content and carbohydrate content of 79.90, 3.83, 8.06, 1.39, 5.92, 0.90% wb, respectively (Mangunwidjaja *et al.*, 2014). While chicory tuber has moisture content, ash content, crude fiber content, fat content, protein content and carbohydrate content of 75.63, 4.25, 5.12, 1.69, 4.65, 8.66% wb, respectively (Massoud *et al.*, 2009).

Table 1 shows that the yacón tuber has a moisture content of 91.23%, higher than the dahlia of 79.9% and the chicory of 75.63% wb. This makes the tuber more susceptible to damage during the harvest season so it needs post-harvest handling such as drying for long-term storage.

Other components in yacón tuber are ash, protein, fat, and crude fiber of 0.21, 0.12, 0.58, and 0.52% wb, respectively. On the other hand, the dahlia tuber has moisture, ash, protein, fat, and crude fiber of 3.83, 5.92, 1.39, and 8.06% wb, respectively. Meanwhile, chicory tuber has a high level of ash, protein, fat, and crude fiber of 4.25, 4.65, 1.69 and 5.12% wb, respectively. Yacón tuber has a lower level of solid constituent besides carbohydrate compared

to dahlia and chicory. Carbohydrate levels indicate the potential for inulin that can be produced because inulin belongs to the carbohydrate class (Apolinário *et al.*, 2014). Yacón tuber has the larger potential to produce an inulin of 7.34% compared to the dahlia (0.90%) but still has not exceeded the potential inulin of the chicory tuber (8.66%).

The purity of inulin isolated from yacón tuber

Inulin can be found in many plants, but it is naturally abundant in chicory roots. It is used widely as low-calorie sugar and fiber replacement in food and beverages (Shoaib *et al.*, 2016). The result of inulin isolated from the yacón tuber was white-yellowish powder with a 4.86% yield (Table 2). The process of isolation of inulin consists of several stages, including washing and stripping, short heating, subsequent precipitation using a mixture of ethanol-water, and the last drying of inulin. The tuber was heated for 1 min at a temperature of 85°C. The detail of all the processes above was very important to prevent the browning process in the yacón tuber. Daulay *et al.* (2017) showed that the tuber contains o-diphenyl derivatives that will undergo an oxidation reaction to make brown color melanoidin compounds. The browning reaction involves enzymatic catechol oxidation or the activity of o-diphenyl oxidoreductase with Cu²⁺ cofactor. Short heating with high temperature was done to inactivate the oxidizing enzyme, so the enzymatic reaction of browning can be prevented. Other types of isolation introduce the use of heat from the microwave oven to enhance the extraction process (Petkova *et al.*, 2018a).

The purity of isolated inulin from yacón tuber was 44.23% (Table 2). The further calculation was done to determine the actual content of inulin by multiplying the percent of the yield and the percent of inulin purity. The actual content of inulin from the yacón tuber

obtained was 2.15%. The yield, purity, and actual inulin levels of inulin in other tubers were obtained from previous research on a dahlia, gembili tuber (Zubaidah and Akhadiana, 2013) and red dahlia (Susilowati *et al.*, 2015). Characteristics of chicory and Jerusalem artichoke were obtained from previous research by Loo *et al.* (1995) which is a plant of commercial inulin production of the world. Inulin yield can be significantly increased by the application of microwave and ultrasound-assisted extractions (Petkova *et al.*, 2018a).

Characterization of isolated inulin

Characterization of isolated inulin was done throughout the Osazon test then compared with chicory inulin, fructose, glucose, and cassava starch as control. The results (Figure 1) showed the crystalline form of glucose and fructose followed with the research of Babu *et al.* (2015) which retested the simple carbohydrate compounds using the Osazon test. Starch crystal created big cotton ball structure, while inulin created smaller cotton ball structure. The isolated inulin has a similar crystal structure with Sigma inulin under the microscope after mixture with Osazon reagents.

Inulin is a carbohydrate polymer, consist of a β-D-fructose monomer linked with an α(2→1) glycosidic bond (Mensink *et al.*, 2015). α-D-Glucose is often found at the end of the polymer (Figure 2). FTIR spectra is a convenient way to characterize carbohydrate based on its functional groups. Interpretation of the results of the FTIR spectrum should be assisted with a correlation table that will indicate the type of function group of the existing wavelength range, this can be applied in the detection of various chemical compounds (Banas *et al.*, 2017).

Table 1. Chemical characterization of tuber

Content in wet basis	Yacón (%)	Dahlia (%) ^a	Chicory (%) ^b
Moisture	91.23±0.02	79.90	75.63±0.39
Ash	0.21±0.00	3.83	4.25±0.11
Protein	0.12±0.09	5.92	4.65±0.25
Fat	0.58±0.00	1.39	1.69±0.71
Crude fiber	0.52±0.02	8.06	5.12±1.55
Carbohydrate	7.34±0.50	0.90	8.66±1.07

Note: ^aMangunwidjaja (2014); ^bMassoud *et al.* (2009)

Table 2. Inulin content in yacón tuber and several other sources

Tuber	Yield (%)	Purity (%)	Actual Content (%)
Yacón	4.86	44.23	2.15
Dahlia ^a	3.22	78.21	2.51
Red dahlia ^b	4.24	57.12	2.42
Gembili ^a	2.32	67.66	1.56
Jerusalem artichoke ^c	21	18.75	3.93
Chicory ^c	24.4	16.2	3.95

Note: ^a Zubaidah and Akhadiana (2013); ^b Susilowati *et al.* (2015); ^c Loo *et al.* (1995)

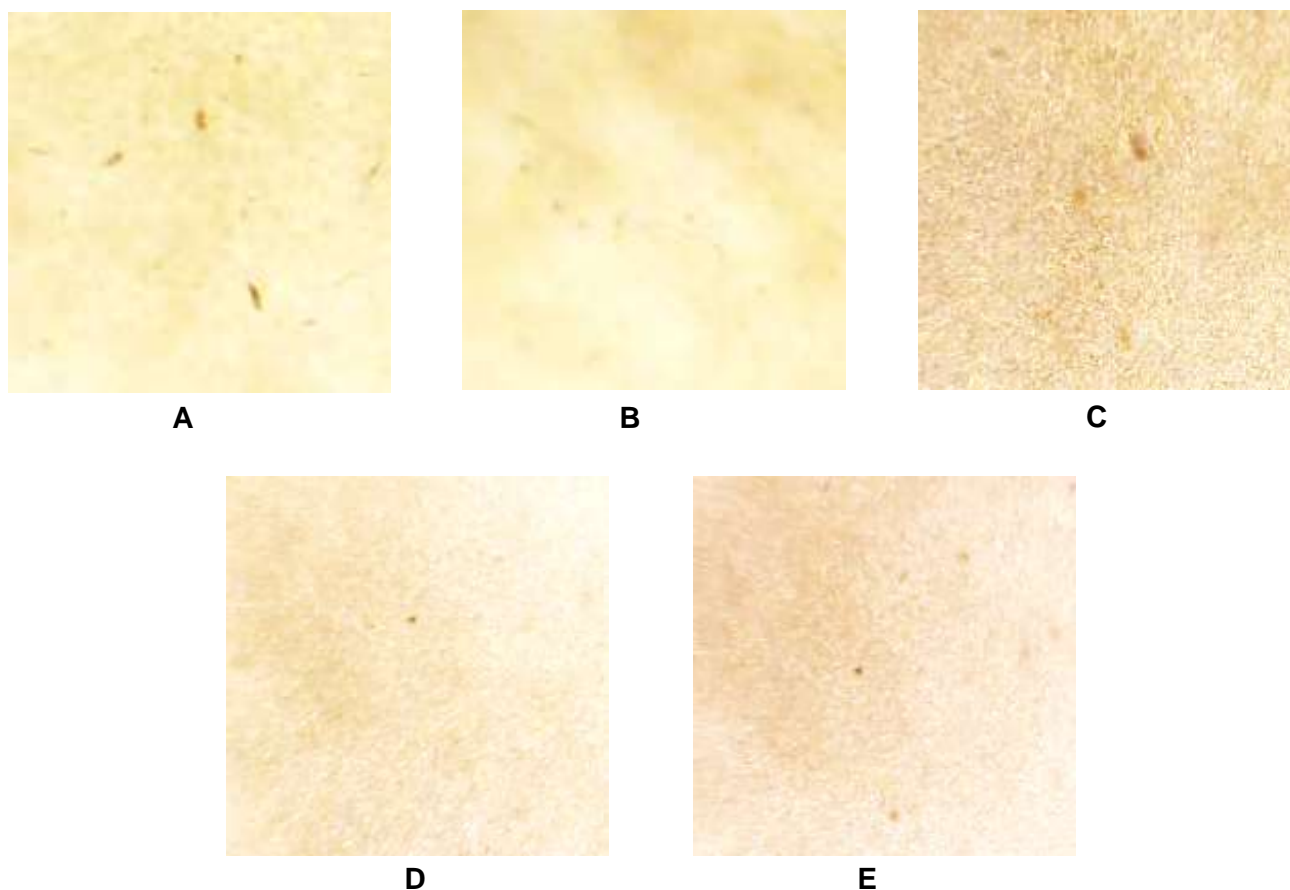


Figure 1. Osazon test result (A) glucose, (B) fructose, (C) cassava starch, (D) chicory inulin, and (E) yacón inulin

FTIR spectrum was compared to the number of wave correlation tables that will show the clusters of functions present in the sample. Characterization of isolated insulin using FTIR was compared with chicory inulin as control. FTIR spectrum chicory inulin (Figure 3) indicates the presence of strong bands on the 1740-1700 cm^{-1} region which is the hallmark of the C=O bond at 1728 cm^{-1} . There are weak to strong bands on the 3400-2400 cm^{-1} area indicating the O-H clusters were at 3348, 3001, and 2399 cm^{-1} . There is a weak band in the area 1300-1000 cm^{-1} which indicates the existence of a C-O group is at 1296 cm^{-1} . There is a medium ribbon in the area 1485-1445 cm^{-1} which indicates the existence of the CH₂ is at 1443 cm^{-1} . Then there is a medium band at 1225-950 cm^{-1} which indicates the existence of a C-H group is at 964 cm^{-1} .

The FTIR spectrum of isolated inulin of yacón (Figure 4) is almost identical to the chicory inulin control. There is a strong band on the 1728 cm^{-1} area which indicates the presence of C=O bonds, medium bands at 3472, 2993, and 2399 cm^{-1} for the O-H clusters. There is a medium band at 1304 cm^{-1} due to the C-O group, the medium band at 1443 cm^{-1} which indicates the presence of CH₂, and the ribbon is at

957 cm^{-1} which indicates the existence of the C-H bond.

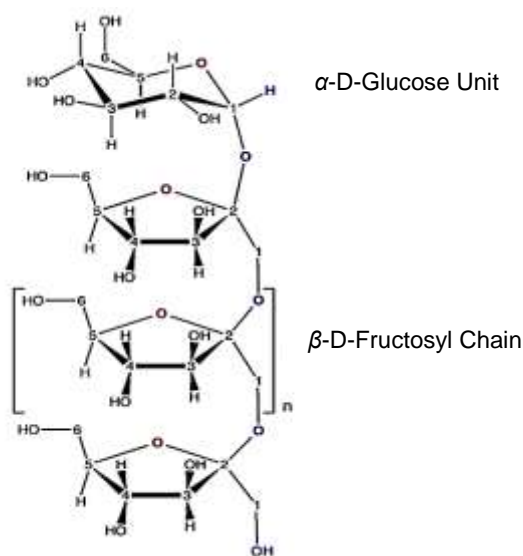


Figure 2. Inulin chemical structure (Barclay *et al.*, 2010)

However, there are medium bands in the area around 3500 cm^{-1} which indicates the presence of amide (N-H) bond at 3533 cm^{-1} . The presence of amide compounds on isolated inulin indicates protein existence, so purification of isolated inulin was needed. The functional group found in the inulin of

chicory and isolated inulin was similar to the results of the characterization of FTIR by Melanie *et al.* (2015). The spectral FTIR inulin of chicory and isolated inulin has a resemblance to the values and shapes.

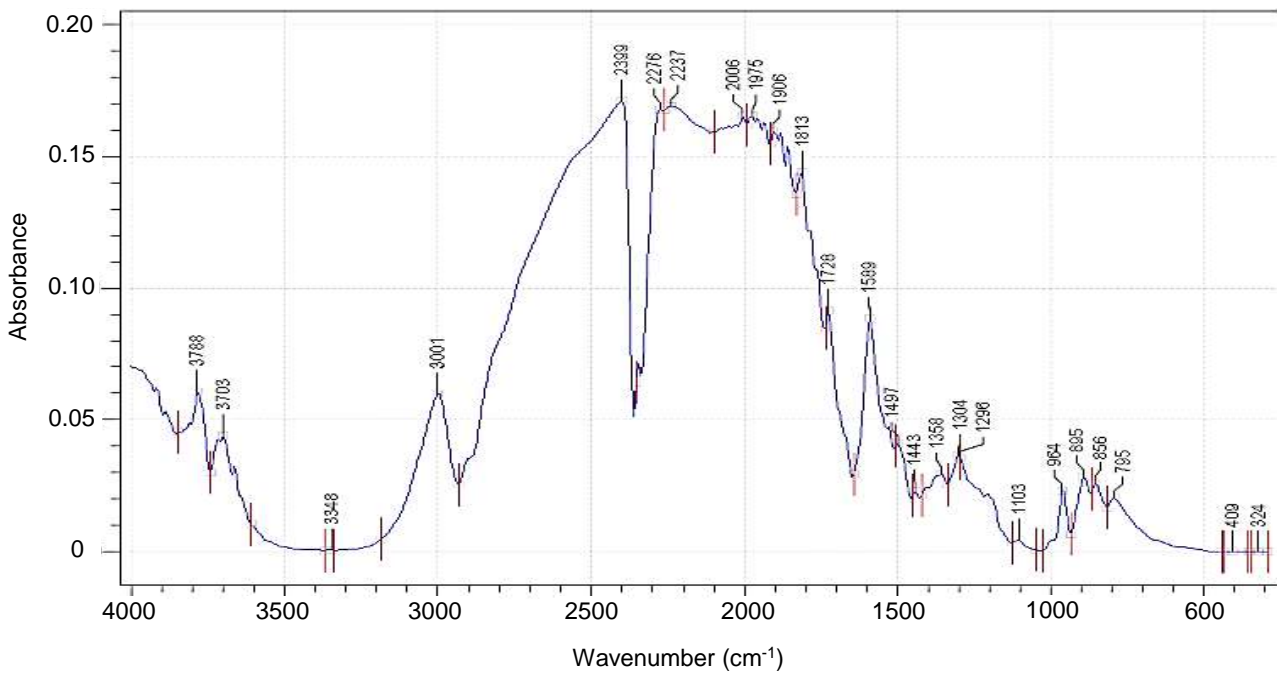


Figure 3. FTIR spectrum of chicory inulin control

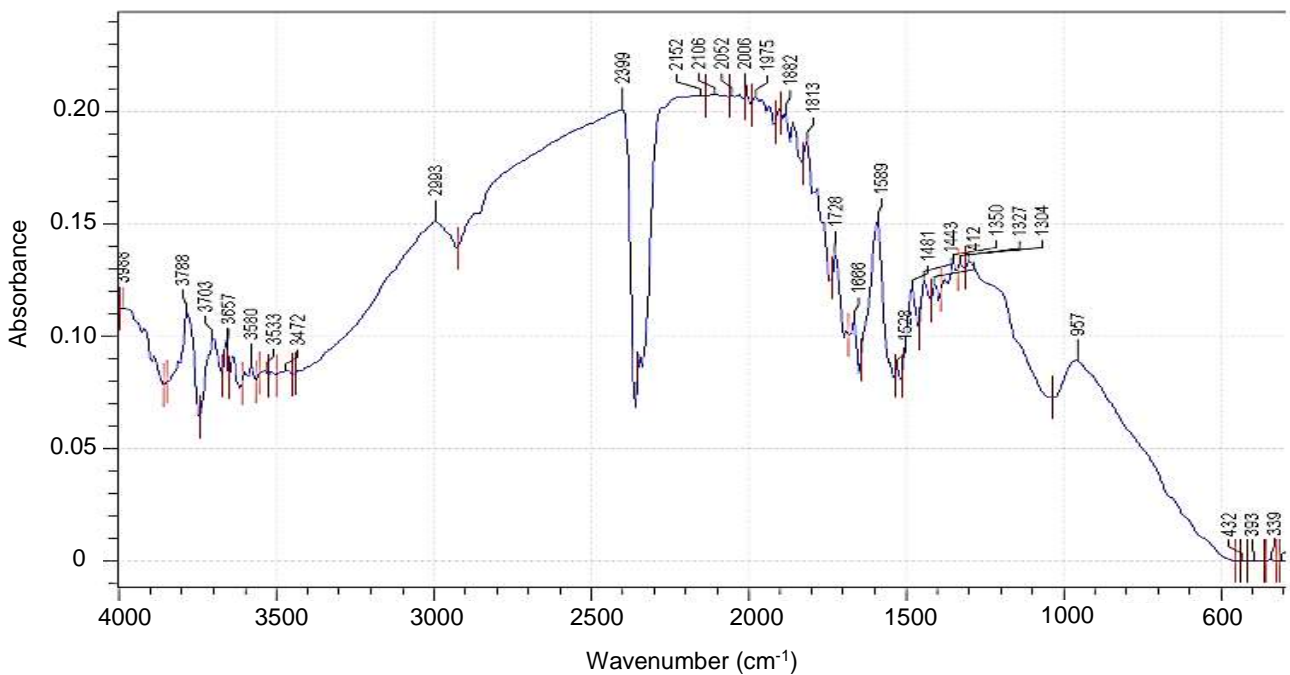


Figure 4. FTIR spectrum of isolated inulin

CONCLUSIONS

Yacón tuber has good potency to produce insulin compare to other local tuber. Isolated inulin of yacón tuber has yield, purity and actual content of 4.86, 44.23, and 2.15%, respectively. Based on crystal structure from Osazon test and FTIR analyses, inulin of yacón share similar structure with chicory inulin. Yacón was evaluated as a source of inulin that is important for food source and nutrition. Thus, this outcome could contribute to the development of inulin production especially in Indonesia.

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