

## Conference Paper

# Isolation and Identification of Inulin and Fos from *Dioscorea Esculenta*

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## Abstract

Inulin and fructooligosaccharides (FOS) OS are dietary fibers that are needed by the body to maintain the health. So far, the inulin used in Indonesia are imported from the other countries. Therefore, this research was conducted to isolate and identify the inulin and FOS from the lesser yam (*Dioscorea esculenta*). In hydrolysis at 30 ° C, pH 5, a concentration of 12% (v / w dried inulin) for 120 hours to obtain the inulin fiber, the crude inulinase enzyme from *Acremonium spCBS3* as the best inulinase type was able to produce SDF (Soluble Dietary Fiber) by 86, 04%.

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## 1. Introduction

Inulin is a polymer of fructose with glucose as the terminal group. Fructose units in inulin are linked by  $\beta$ -(2 $\alpha$ 1)-glycosidic. As the soluble dietary fiber, inulin is useful for digestive system as well as the body health (Sardesai, 2003). Inulin is soluble in water, but it could not be digested by enzymes in mammal digestive system. Inside the colon, inulin was fermented by the bacteria., so it has the positive impact to the health. Some of the type of *Bifidobacteria* can use inulin as the source of energy because they produce an extracellular cellulose that can hydrolyze the bond of  $\alpha$ -(2-1)-D-fructose to fructose (Robertfroid, 2005).

Inulin is widely used in the food industry, for example, as a component (ingredient) of various types of food products. Inulin needs in Indonesia increase from year to year. However, all the inulin needs are still fulfilled by the imported inulin. The volume and value of inulin imports in 2008 was 1,420,522 kg with a value of 4,664,245 US \$ and in 2010 was 4,021,679 kg with a value of 13,190,242 US \$. Kg (Central Bureau of Statistics, 2012). Therefore, it is necessary to find inulin sources from local raw materials to reduce the dependence of inulin from other countries. One local raw materials containing inulin is *Dioscorea* spp. tuber with the inulin content varies between 2.88% -14.77%. The highest inulin content is found in *Dioscorea esculenta* (lesser yam) by 14.77% (Winarti et al., 2011).

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Lesser yam (*Dioscorea esculenta*) is one of the most widely grown species in many areas of Indonesia. They grow wild in the yards and in the forests. The tuber of this plant is usually used by the people as an alternative source of carbohydrates, but until now the utilization is still very limited. Isolation of inulin from lesser yam is one of the efforts and new innovations to utilize the abundant local natural resources.

## 2. Methods

### 2.1. Inulin isolation from the lesser yam

Isolation of inulin was conducted according to the procedure used by Park et al. (2006) and Toneli et al. (2008) with some modifications. The lesser yam was cleaned, washed, peeled and cut into small pieces, then blended with the addition of hot water in temperature of 80-90 ° C 1:10 (tuber: water). The next was diffusion in a waterbath shaker at 90 ° C for 1 hour. After being filtered and cooled, it was then frozen at -20 ° C for 24 hours. The frozen filtrate was thawed and then centrifuged at 1500 rpm for 15 minutes to obtain the white precipitate which was then separated. The white precipitate was then dried using a dryer cabinet at 60 ° C for 5 hours, mashed and sieved.

### 2.2. Isolation and identification of FOS from Gembili

Application of the use of the inulinase enzyme in inulin hydrolysis for the acquisition of dietary fiber is performed on the mold with the best inulinase activity. Inulin produced from lesser yam extraction (ratio= 1 tuber: 2 water) was through the process of gelatinization at 85-90 ° C for 30 min, then filtration pass of 80 mesh, sedimentation using 50% ethanol, and PH regulation to pH 10 (Susilowati et al., 2012). Then the formulation was added by a rough inulinase enzyme from *Acremonium* sp-CBS3 at a concentration of 12% (v / b inulin) at pH 5.0, for comparison was the formulation without inulinase enzyme (0%). The suspension was further heated in a shaker with agitation at a rotational speed of 160 rpm for 120 h at 30 ° C to obtain hydrolysate as an inulin fiber.

## 3. Results and Discussion

### 3.1. The purity of inulin

Inulin from lesser yam has the same retention time as the standard inulin (inulin SD) of 5.212 minutes. In Figure 1, it can be seen that the area of standard inulin is 100%, while

the fM inulin is 85.828%. Based on the area, the purity of fM inulin can be calculated, that is 66.340%. The standard inulin used was inulin isolated from chicory tubers with a molecular weight of 990.8. If the inulin retention time of lesser yam is the same as for standard inulin, then it is assumed that the molecular weight of the lesser yam inulin is equal to the standard inulin. Based on the inulin molecular weight, the amount of inulin polymer can be calculated as follows:

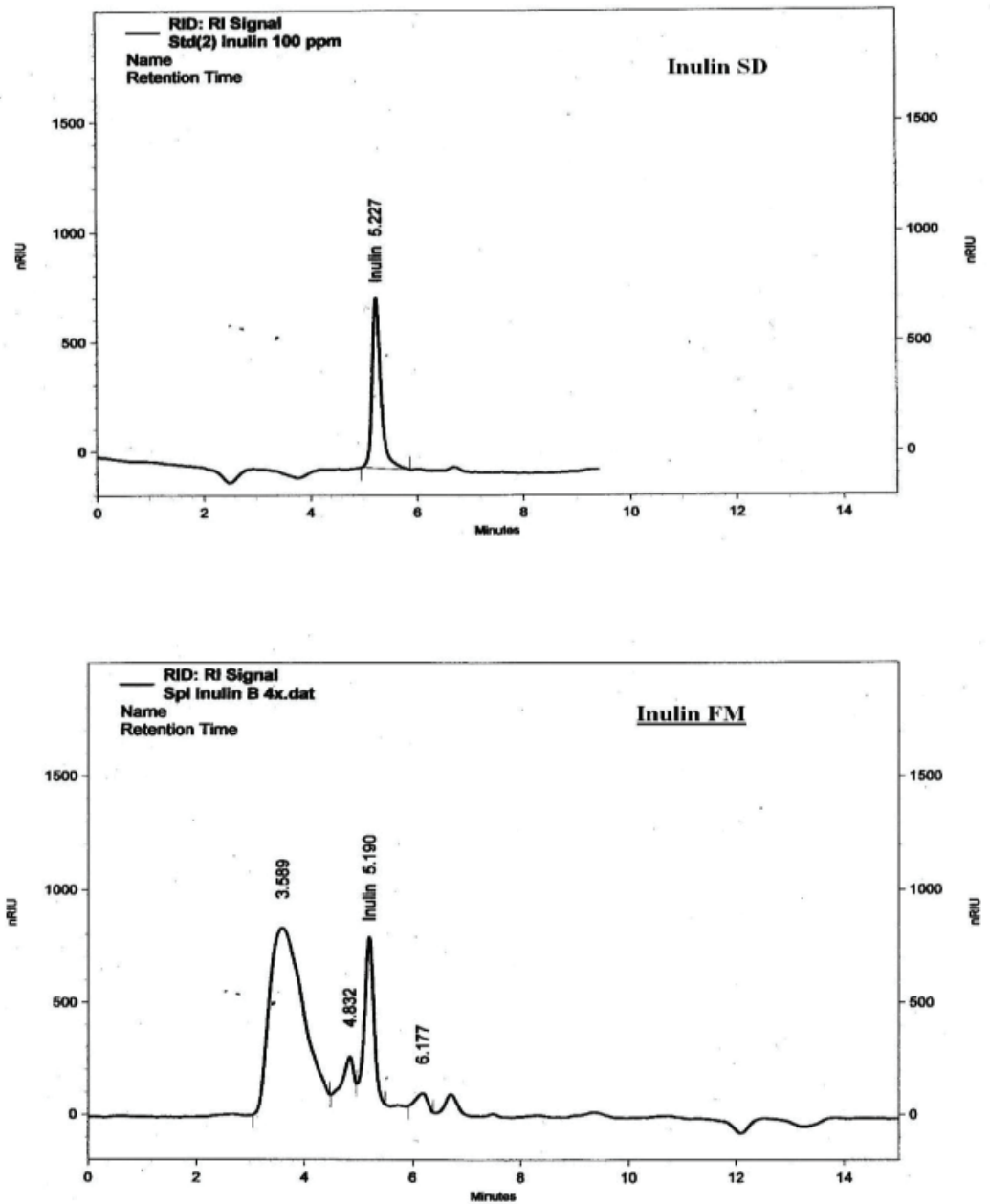
$$\begin{aligned} 990.8 \text{ (BM inulin)} &= (\text{C}_6\text{H}_{10}\text{O}_5)_n + \text{H}_2\text{O} \\ &= (162)n + 18 \\ 162 n &= 990.8 - 18 \\ n &= 6.0049 \end{aligned}$$

So, the degree of polymerization (DP) of lesser yam inulin is 6.

### 3.2. Fructo Oligosaccharide (FOS)

The application was carried out by the type of mold with inulinase activity i.e. *Acremonium* sp-CBS3 through the inulin hydrolysis of the lesser yam at pH 5, 12% crude inulinase enzyme concentration (v / b inulin dry), agitation of 160 rpm, for 120 hours at 30°C with comparable starting materials of inulin without hydrolysis process. The inulin composition as the substrate shows the total solids of 4.239%. Reduced sugar of 2.0 mg / mL, inulin of 57.12% (dry weight), total sugar of 1.0 mg / mL, SDF of 1.84% (dry weight) and IDF of 5.817% (dry weight) were obtained from this treatment. The inulin hydrolysis process using a crude inulinase enzyme from *Acremonium* sp-CBS3 (12% w / v inulin) yielded a hydrolyzate with a total solids composition of 3.983%. Sugar reduction of 3,3458 mg / mL, inulin of 19,9098% (dry weight), total sugar of 2.0667 mg / mL, SDF of 13,182% (dry weight) and IDF of 5,9019% (dry weight).

The content of SDF, IDF, reduced sugars and total sugars obtained is due to the inulinase activity in inulin hydrolysis which is influenced by environmental factors (pH, temperature, hydrolysis time). *Acremonium* sp. enzyme concentration has the best hydrolysis condition at 30 ° C, pH 5 even though it also has the activity of glucoamylase and  $\alpha$ -amylase enzymes under the same conditions (Marlida, 2001), so that it is possible that this increase is not totally caused by inulinase enzyme. Changes in the composition of SDF, IDF, reducing sugars and total sugars lead to decreased inulin and total solids. The decrease in total solids is caused by process conditions, such as temperature and time (30 ° C, 120 hours) which leads to a higher level of solubility of all components. In addition, the decrease in inulin also can be caused by some inulin that has been



**Figure 1:** Comparison between the purity of lesser yam inulin with *foam mat drying* technique (Inulin fM) and commercial inulin (Inulin SD).

hydrolyzed into sugars as SDF and IDF. However, not all inulin can be hydrolyzed with this enzyme and leaving 65.14% of the total inulin after the hydrolysis. These results are also affected by the level of purity of inulinase as well as the environmental factors during the hydrolysis.

## 4. Conclusion

The purity of Inulin from lesser yam is 73.585%. In the hydrolysis of inulinase enzymes on lesser yam inulin in a temperature of 30 ° C, ph 5, concentration of 12% (v / b inulin dried) for 120 hours for the inulin fiber gain, the crude inulinase enzyme from *Acremonium* sp-CBS3 can increase the SDF (Soluble Dietary Fiber) by 86.04% of the initial SDF inulin (1.84% b.k.) to SDF in inulin hydrolyzate (13.182% bk).

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