The Effect of Solvent Ratio and Precipitation Time on Isolation of Inulin from White Sweet Potato (Ipomoea batatas L.)

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Abstract. White sweet potato has the potential as a source of inulin and is largely abundant in Indonesia. The solvent ratio and precipitation time in ethanol solvent can increase yield produced in the inulin isolation process. This research aimed to determine the effect of different solvent ratios and precipitation times on inulin yield in the extraction-isolation process and as well to know the physical and chemical characteristics of white sweet potato inulin. The stages of the study consisted of determining the total sugar content and sugar reduction, extraction, isolation, physical and chemical characterization. This research used Factorial Completely Randomized Design (RALF) with two factors, different solvent ratio were 1:1, 1:2, and 1:3 with precipitation time for 6, 12, and 18 hours. Data were analyzed used General Linier. The analysis was followed by DMRT (α = 0,05). The results showed that the ratio of 1:2 and the precipitation time of 12 hours produced the best inulin yield of 7.72%. It had an effect on the physical characteristics of inulin that were produced, namely color, solubility, water absorption and water content. While the other characteristics in the form of ash content were not significantly different in ratio and precipitation time.

1. Introduction
Inulin is a functional compound that can provide physiological functions and beneficial for health. Inulin belongs to polysaccharides group consisting of a straight D – Fructose chain with one unit of glucose at each end [1]. Inulin has benefits as prebiotic and can be added to fat substitute food products [2]. Inulin is found in many roots and stems of tubers. Inulin production in Indonesia itself is still limited to the commercial tubers of Chicory and Jerusalem Artichoke. There have been many studies on the development of inulin in various foods such as dahlia tubers, various kinds of yams, bengkoang, shallots, and dandelion plant roots. One of the plants in Indonesia that has the potential to contain inulin and its abundant availability is sweet potato.

Previous research by Afriani (2016), she conducted a research about inulin testing on several sweet potato varieties, namely white, purple and yellow sweet potatoes [3]. However, in those studies, extracted inulin was only seen physically outside and has not been tested for the presence of inulin based on functional groups. In addition, there has been no further research regarding the characterization of inulin derived from sweet potatoes.

In this study, further research on inulin of white sweet potato was conducted in which research by Afriani (2016) has not been carried out on isolation or characterization of inulin. The aimed of the study was carried out through several stages including extraction, isolation, and characterization using solvents with different ratios and precipitation times. The characterization of inulin was performed using FTIR to determine the presence of inulin based on functional groups.
2. Materials and methods

2.1 Materials

The main material used in this study was the Sukuh, the Sukuh is a variety of sweet potato locally grow in Karanganyar Regency, and variety of white sweet potato tuber with a 4.5–5month harvest age obtained from Pasar Gede, Surakarta. Other materials used were concentrated H₂SO₄, pure phenol, Na₂SO₃, NaOH, glucose, distilled water obtained from the Biochemical Laboratory of Food Science and Technology Study Program Sebelas Maret University (UNS) and DNS reagents, Rochelle salt, and ethanol 95% obtained from Universitas Islam Negeri Yogyakarta.

2.2 Extraction, Isolation, and Characterization of White Sweet Potato Inulin

This research was based on the modified method from Kosasih et. al., including extraction preparation, analysis of total sugar and sugar reduction, extraction, isolation, and characterization [4]. After milling the ingredients into flour, then an analysis of the total sugar and its reduction was carried out to determine the presence of potential inulin in the sample. Then it was followed by extraction in order to obtain the inulin. During isolation process, inulin extract was treated in the form of variations using a solvent ratio of 1: 1, 1: 2, and 1: 3 with the time of precipitation of 6 hours, 12 hours, and 18 hours. The obtained Inulin extracts were physically characterized namely yield, color, solubility, and water absorption and chemically including water content, ash content, and functional group analysis using the FTIR spectrum.

2.3 Physical and Chemical Analysis

The analysis focused primarily on the total amount sugar using Phenol-Sulfuric Acid method [5], and then sugar reduction using Dinitrosalicylic Acid method [6], yield measurements using the inulin isolation extraction method [4] [7], and color using the chromameter method [8], solubility using analysis of solubility in water method [7], water absorption using the analysis of inulin water absorption method [7], water content using the thermogravimetric method [9], ash content using the dry ashing method [7], and functional group analysis using the Forier Transform Infrared (FTIR) spectrum [10]. All samples compared with Orafti® GR standard.

2.4 Data Analysis

The data were analyzed using General Linear Model method. Duncan's Multiple Range Test (DMRT) was considered to test the significance level at 5% (p ≤ 0.05) when difference in treatments was noticed.

3. Results and discussion

3.1 Total Sugar and Sugar Reduction

| Type of Analysis          | Sample Content (%) | Sample Reference Content (%) |
|--------------------------|--------------------|------------------------------|
| Total Sugar              | 9.81 ± 0.007       | 9.533–17.258 [11]            |
| Sugar Reduction          | 2.74 ± 0.064       | 2.77 [10]                    |

Table 1 shows the total sugar content of white sweet potato was 9.81% and sugar reduction was 2.74%, so the remain sugar content was 7.07%. The total sugar content was in accordance with Bouaziz et. al., it stated that the total sugar content in sweet potatoes ranged from 9.533-17.258% [11]. While the reducing sugar content was not much different from a research by Apolinário et al., it was 2.77% [10]. Based on non-reduced sugar levels, it can be seen that there was a potential for inulin content of below 7.07% and it can be continued with extraction, isolation, and characterization to determine the presence of inulin groups. When testing both the reducing and
non-reducing sugar content in white sweet potato flour, it indicated the presence of inulin in the samples. According to Glibowski and Bukowska, if the reducing and non-reducing sugar content exceed the standard, it would be damaged inulin [12].

### Table 2 Effect of Solvent Ratio and Precipitation Time on Yield, Color, Solubility, and Water Absorption of White Sweet Potato Inulin

| Treatment                  | Yield (g/100g) | Whiteness | Solubility (%) | Water Absorption (%) |
|----------------------------|----------------|-----------|----------------|----------------------|
| **Solvent Ratio (%v/v)**   |                |           |                |                      |
| 1:1                        | 6.377 ± 0.180^b | 81.514 ± 0.975^a | 5.544 ± 0.256^a | 37.723 ± 2.048^a |
| 1:2                        | 6.717 ± 0.783^c | 82.066 ± 1.063^b | 5.754 ± 0.349^b | 39.993 ± 2.713^b |
| 1:3                        | 6.092 ± 0.272^a | 82.299 ± 1.141^c | 5.983 ± 0.237^c | 40.110 ± 3.478^c |
| **Precipitation Time (hours)** |         |           |                |                      |
| 6                          | 6.389 ± 0.120^b | 80.593 ± 0.233^a | 5.528 ± 0.255^a | 36.569 ± 1.287^a |
| 12                         | 6.847 ± 0.662^c | 82.334 ± 0.560^b | 5.718 ± 0.204^b | 38.108 ± 1.445^b |
| 18                         | 5.949 ± 0.203^a | 82.952 ± 0.322^c | 6.036 ± 0.313^c | 42.153 ± 2.203^c |

Notes
- Solvent ratio = inulin extract: ethanol
- Different letters in the same column for each treatment show a significant difference (p<0.05)

3.2 Extraction and Isolation of White Sweet Potato Inulin

Based on Table 2, it can be seen that the variation in the ratio of solvents of 1:2 resulted into a significant increase in inulin yield. However, the treatment ratio of 1:3 resulted into yield decrease. This result was in accordance with the study of Kosasih et al. in Dahlia tuber. It stated that the ratio of inulin extract and ethanol 1:2 produced the highest inulin at 9.03% [4]. This could be caused by the nature of ethanol to inulin. It makes difficult to dissolve but quicken the process. However, too much ethanol can extract or precipitate other compounds other than inulin [13].

Variation in precipitation time produced a significant difference (p<0.05) to the yield of inulin in white sweet potato. The precipitation time of up to 12 hours resulted in the best white sweet potato inulin yield. These results were slightly different from a research by Mezoti et al. in dahlia tubers, which the optimum deposition time in producing the highest inulin was 6 to 24 hours and after that the inulin content will decrease [13]. This means that white sweet potatoes do not need to be precipitated up to 24 hours to produce maximum inulin. The interaction of the solvent ratio and precipitation time had a significant effect on the yield of inulin in white sweet potato (p<0.05).

The best white sweet potato inulin yield was obtained by variations of solvent ratio of 1:2 and precipitation time of 12 hours which was 7.72%. The inulin yield value was greater when compared to research by Afriani, which has the highest yield value of 5.5% [3]. According to Azhar, the use of ethanol was effective for precipitating inulin because the nature of ethanol is difficult to dissolve in inulin [14]. Precipitation of inulin using ethanol was related to the polymerization degree (DP) of inulin in the sample. Ethanol solvents will precipitate inulin with a high polymerization degree first, which was DP 26. The higher the solvent ratio and the more precipitation time, the more inulin deposits with high, medium and short DP variations, the more yield will be produced [15].

3.3 Physical Properties of White Sweet Potato Inulin

Based on Table 2 it can be seen that the variations of solvent ratio and precipitation time gave a significant difference (p<0.05) to the whiteness of white sweet potato inulin. The increase in solvent ratio and precipitation time significantly resulting in higher whiteness values and can be concluded that the produced inulin becomes much whiter. This is related to carotenoids in the white sweet potato sample. According to Lorenz, ethanol was very good at dissolving B-carotene [16]. In addition, carotenoids are hydrophilic, lipophilic, insoluble in water, soluble in acetone,
alcohol, and chloroform solvents so that carotenoid pigments dissolve producing white inulin [10]. The interaction of solvent ratio and precipitation time gave a significant effect on the color or whiteness of white sweet potato inulin (p <0.05). The best white sweet potato inulin color was obtained by varying the solvent ratio of 1:3 and 18 hours precipitation time.

Variations in solvent ratio gave a significant difference (p <0.05) to the solubility of white sweet potato inulin. This is related to the precipitation and the polymerization degree of inulin. According to Dewi, solubility of inulin is influenced by the polymerization degree (DP) contained in inulin [17]. The higher the polymerization degree, the more difficult it would be for inulin to be dissolved in water [17]. High solvent ratio causes inulin deposits with varying polymerization degree to be smaller and settling more and more. The amount of inulin with small and medium polymerization degree that settles caused water to enter easily and solubility to rise [18].

Variation in precipitation time gave a significant difference (p <0.05) to solubility of white sweet potato inulin. High solubility was associated with free OH groups in the sample after ethanol administration. Ethanol has a free OH group in its compound chain. The longer the precipitation time with ethanol, the more free OH groups in the sample mixture to increase, the increase in solubility of inulin was caused by hydroxyl (OH) groups making it easily soluble in water [19]. The interaction of solvent ratio and precipitation time gave a significant effect on the solubility of white sweet potato inulin (p <0.05). The overall sample of white sweet potato had a solubility value of 5.761%. This value was under the Orafti® GR standard which was 7.59% and Jerusalem artichoke was 6.99–8.07% [10].

Variation in precipitation time gave a significant difference (p <0.05) on water absorption of white sweet potato inulin. Similar to the solubility value, water absorption was related to the precipitate formed and the polymerization degree of inulin. High solvent ratio caused inulin deposits in various polymerization degrees into small, medium, and high settling more and more. The amount of inulin in small, medium, and high polymerization degree that settles caused solubility to rise, and the water absorption to rise [18].

Variation in precipitation time gave a significant difference (p <0.05) on water absorption of white sweet potato inulin. Similar to solubility value, high water absorption was related to the free OH group in the sample after ethanol administration. Ethanol had a free OH group in its compound chain. Long precipitation time with ethanol caused the freed OH group in the mixed sample to increase [19]. The interaction of solvent ratio and precipitation time gave a significant effect on the value of water absorption of white sweet potato inulin (p <0.05). The value of water absorption of white sweet potato inulin has an average of 38.94%. This value was below Dahlia tuber, which is more than 40% [7]. However, when compared with Orafti® GR standard and Jerusalem artichoke tubers the value of absorption of white sweet potato water was higher, which was 12.2%; 5.8–72% [10].

| Table 3 Effect of Solvent Ratio and Precipitation Time on Water and Ash Content in White Sweet Potato Inulin |
|---------------------------------------------------------------|
| **Treatment** | **Water Content (%)** | **Ash Content (%)** |
| Solvent Ratio (%v/v) | 1:1 | 0.556± 0.019<sup>a</sup> | 7.598± 0.044<sup>a</sup> |
| | 1:2 | 0.559± 0.030<sup>a</sup> | 7.645± 0.063<sup>b</sup> |
| | 1:3 | 0.564± 0.030<sup>a</sup> | 7.687± 0.048<sup>c</sup> |
| Precipitation Time (hours) | 6 | 0.551± 0.023<sup>a</sup> | 7.601± 0.047<sup>a</sup> |
| | 12 | 0.557± 0.027<sup>a</sup> | 7.634± 0.047<sup>b</sup> |
| | 18 | 0.573± 0.023<sup>a</sup> | 7.694± 0.058<sup>c</sup> |

Notes
- Solvent ratio = inulin extract: ethanol
- Different letters in the same column for each treatment show a significant difference (p<0.05)
3.4 Chemical Properties of White Sweet Potato Inulin

Variation of solvent ratio gave a significant difference (p <0.05) to the water content of white sweet potato inulin. High solvent ratio caused inulin deposits in various polymerization degrees into small, medium, and high settling more and more. The polymerization degree of small and medium inulin that settles causing the solubility to rise, and water gets higher the water content gets higher [18].

Variation in precipitation time gave a significant difference (p <0.05) to water content of white sweet potato inulin. Ethanol had hygroscopic properties so it easily absorbs water. The longer the inulin solution was in contact with free air, the easier water enters hence the water content gets higher [17]. The interaction of solvent ratio and precipitation time gave a significant effect on water content of white sweet potato inulin (p <0.05). The overall sample had an average of 7.643% which means that it had a dry matter content of 92.315%. The results were not much different from the inulin standard of Chicory tubers with dry matter content of 91.67% [12] and Dahlia tuber with dry matter content of 92.2% [7].

Based on Table 3, it can be seen that the variations of solvent ratio and precipitation time did not give a significant difference (p <0.05) to ash content of white sweet potato inulin. The interaction of solvent ratio and precipitation time with ash content of white sweet potato inulin was not shown because it had no effect (p <0.05). Ash content of white sweet potato inulin was in ranged from 0.546 to 0.579%. The ash content was in accordance with the fibruline standard of Chicory tuber which is 0.5–0.63% [20][7].

Figure 1 FTIR Infrared Spectrum (a) Commercial Inulin (b) Inulin with solvent ratio 1:2 for 12 hours

Based on Figure 1, the absorption number and results of the FTIR infrared spectrum of white sweet potato inulin samples were not much different from commercial inulin [2]. The results of FTIR spectrum can be seen that all absorption numbers of white sweet potato samples were in the range of functional group wave uptake which indicates the presence of inulin in the sample. The hydroxyl (OH) group shows the main characteristics of inulin [2]. This hydroxyl group was in the absorption range between 3550–3230 cm⁻¹ and had an asymmetrical bonding band. Carbohydrates are shown in the absorption band of carbonyl groups (C = O) in the absorption range between 1470–1430 cm⁻¹.

4. Conclusion

The difference in ethanol solvent ratio and precipitation time in white sweet potato inulin isolation process gave a significant effect on the physical properties of inulin in the form of yield, color, solubility, and water absorption. The best white sweet potato inulin ratio was on ratio 1:2 with a 12-hour precipitation time of 7.72%. Chemical properties had a significant effect on water content while ash content does not. Functional group analysis using FTIR spectrum proved the presence of inulin in white sweet potatoes.
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