Natural Inhibitors to Increase the Antioxidant Activity of Yacon Tubers Syrup

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Abstract. Yacon tubers contain various bioactive including flavonoids and phenolic compounds, having antioxidant properties. The aim of the study was to obtain the effect of using natural inhibitors on the antioxidant activity of yacon tubers syrup. Phytochemical tests are used in the determination of flavonoids, phenolic content and antioxidant activity. Total content of phenol and flavonoid were measured by spectrophotometry, while the antioxidant activity was measured by DPPH method. The results showed that the use of natural inhibitors affects the antioxidant activity of yacon syrup. The content of flavonoids and phenolic compounds in yacon syrup using natural inhibitors were 148.093±3.973 and 1244.595±67.672 ppm, significantly different from yacon syrup without inhibitors 100.923±9.277 and 978.912±54.223ppm. Antioxidant activity of syrup with natural inhibitors were 52.888% and IC50: 6.383, while yacon syrup without inhibitors were 24.388% and IC50: 11.180.

1. Introduction
Yacon [Smallanthus sonchifolia (Poepp.et Endl.) H. Robinson] tuber plants originating from the Andes mountains. Yacon has the potential to be well developed, its tuber as an anti-diabetic sweetener, anti-hypercholesterolemic. Besides fructooligosaccharides (FOS), yacon tubers also contain secondary metabolites of phenolic compounds including flavonoid compound. It stimulates immune system through their effects as antioxidants, anti-inflammatory, anti-microbial and anti-cancer properties [1]. It has been reported that phenolic and flavonoid compounds act as antioxidants [2]. The tubers contain FOS as the storage saccharides recommended as prebiotics, but phenolic compound have also been isolated from tubers. It has been found that yacon contains a considerable amount of phenolic compounds, as much as 3.8% on dry weight basis [3].

Phenolic compounds are referred to as phytochemicals. As natural dietary antioxidants, phenolic compound from plants may protect cell membranes against damage by oxygen radicals. Phenolic compounds comprise one (phenolic acids) or more (polyphenols) aromatic rings with attached hydroxyl groups in their structures. Their antioxidant capacities are related to these hydroxyl groups and phenolic rings. As the major water-soluble phenolic constituents, five caffeic acid derivates were isolated from yacon tubers, mainly chlorogenic acid and 3,5-dicaffeoylquinic acid and three esters of caffeic and altraric acid [4]. Polyphenol compounds of extracts from yacon tubers such protocatechuic, chlorogenic (CA), caffeic, and ferulic acids contribute a great deal to the antioxidant activity [5].

Polyphenols are well-known, pharmacologically active compounds with immunomodulatory activity. This category includes flavonoids, phenolic acids, and stilbenoids, which are ubiquitously
produced in plants and exist either as free aglycones or in a state of esterification with glucose and other carbohydrates (glycosides). Consequently, the absorbed polyphenols interact with the intestinal immune system, leading to both protective and harmful reactions in the host. Current evidence strongly suggests that polyphenols contribute to the prevention of several immune diseases. For example, polyphenols in red wine can significantly increase the level of interleukin (IL-21) and decrease the release of IL-1β and IL-6[6].

Fresh yacon tubers are highly perishable and tend to quickly brown during post-harvest handling and processing. One option to prolong shelf-life of yacon tubers is to process them into syrup. During dehydration, enzymatic activity can be inhibited by heating and addition of inhibitors. Accordingly the effect of using natural inhibitors must be obtained in the manufacture of yacon tubers syrup to maintain the bioactive component of yacon tubers, especially phenolic compounds that function as antioxidants. In the present study, we determined the effect of using natural inhibitors on the manufacture of yacon tubers syrup on total phenolic levels, flavonoids and antioxidant activity.

2. Method

2.1 Instrumentation
The study used equipments involving blander, knife, stove, glasses, spatula, thermometer, pH meter, and spectrophotometer.

2.2 Materials
Materials, were processed in the research, were yacon tubers, ascorbic acid, citric acid, and DPPH (1.1-diphenyl-2-picrylhydrazyl).

2.3 Procedures
Yacon tubers obtained from Wonosobo are divided into two groups of syrup manufacturing processes, with natural inhibitors and without inhibitors. The use of natural inhibitors is carried out by soaking the yacon tubers in a solution of ascorbic acid and citric acid. Evaporation temperature and pH were controlled at ± 65 °C, pH 5-6 for syrup with inhibitors and pH 6-7 without inhibitors. Total phenolic content was determined using the Folin-Ciocalteu method, absorbance was measured at 756.2 nm after 2 hours using a spectrophotometer, and flavonoids with quercetin as standard were measured at 437.9 nm. The antioxidant activity of syrup was evaluated by DPPH (1.1-diphenyl-2-picrylhydrazyl) assay. An independent t test was applied (α = 5%) to determine differences of phenolic compounds, flavonoids contents and antioxidant activity between yacon syrup with and without natural inhibitors.

3. Results and Discussion
Preparation of yacon syrup without inhibitors produces a greenish black syrup while the inhibitor is brownish yellow. Natural inhibitors used are able to inhibit the browning process that occurs so that a yellow slurry is obtained and the evaporation process will produce a brownish yellow color. Various compounds can be a substrate for the browning process, including chlorogenic acid, caffeic acid and tyrosine. Orthodihydroxy or trihydroxy phenolic compounds that are close to each other are good substrates for the browning process.

The browning process is also caused by enzyme activity against phenolic compounds, polyphenols, chlorogenic acid, which ends with hydroquinone polymerization and produces a brown color. This reaction depends on the polyphenol oxidase enzyme with copper and O2 cofactors as electron donors. The addition of citric acid as a chelating agent will chelate copper (Cu) resulting in enzyme inactivation; while ascorbic acid as reducing agent will reduce quinone to chlorogenic acid again. The reaction of changing chlorogenic acid to form quinones is as follows [7].

\[
\text{Chlorogenic acid} + \text{[O]} \rightarrow \text{quinone (by enzyme)} \\
\text{Quinone} + \text{H}_2\text{O} \rightarrow \text{hydroxychlorogenic acid} \\
\text{Quinone} + \text{hydroxychlorogenic acid} \rightarrow \text{hydroxyquinone} + \text{chlorogenic acid} \\
\text{Chlorogenic acid} + \text{[O]} \rightarrow \text{quinone (by enzyme)} \\
\text{Hydroxyquinone} \rightarrow \text{polymer}
\]
The total phenolic compound content of yacon syrup without or using natural inhibitors was measured by the Folin-Ciocalteu method based on gallic acid standards and flavonoid levels with quercetin standards. The data are in Table 1. The independent t test results on the total phenolic and flavonoid levels in Table 1, show that there is a significant difference (p = 0.000).

### Table 1. Average of total phenolic and flavonoid compound content of yacon syrup

| Treatments | Average of total phenolic (ppm) | Average of total flavonoid (ppm) |
|------------|---------------------------------|----------------------------------|
| (-) inhibitor | 1008.96 | 111.718 |
|             | 973.248 | 106.934 |
|             | 1050.56 | 94.662  |
|             | 954.144 | 88.694  |
|             | 907.648 | 102.606 |
| average     | 978.912±54.223 | 100.923±9.277 |
| (+) inhibitor | 1356.384 | 144.78 |
|             | 1249.024 | 150.038 |
|             | 1177.376 | 142.982 |
|             | 1213.664 | 152.132 |
|             | 1226.528 | 150.532 |
| average     | 1244.595±67.672 | 148.093±3.973 |

Sign. (p) 0.000

Yacon-inhibited syrup has higher levels of total phenolic compounds and flavonoids than yacon syrup without inhibitors; means that the use of natural inhibitors can maintain or increase the total levels of phenolic compounds and flavonoids in the manufacture of yacon syrup. Natural inhibitors are used through denaturation temperature regulation, use of reducing agents and chelating agents to maintain levels of flavonoids and phenolic compounds. Besides the effect of natural inhibitors on plant cell wall membranes, the natural inhibitors used can inhibit enzyme activation and degradation reactions that change phenolic compounds and flavonoids in the manufacture of inhibited yacon syrup. The use of acid as a natural inhibitor is also able to denature the plant cell membrane so that more vacuole cell walls are broken so that phenolic compounds including flavonoids can be extracted.

Various studies have shown that heating will reduce levels of phenolic compounds and flavonoids; among others, Ghafoor et al [8] found that the heating process of plums and mahaleb will significantly reduce the total levels of phenolic compounds. Flavonoids are a group of polyphenols with a basic phenol structure whose compounds are easily oxidized and sensitive to heat treatment so that the cooking stage will affect the levels. According to Qiao et al [9], flavonoid compounds are sensitive to heat because of their hydroxyl and ketone groups, as well as unsaturated double bonds. At high temperatures, the flavonoids is unstable. It is due to oxidation reactions that break the conjugated double bonds.

Adding an inhibitor can lower the pH of the yacon syrup. The original pH of yacon tuber slurry without inhibitor was pH 6-7, with immersion in ascorbic and citric acid inhibitor will produce slurry or yacon syrup with a lower pH, namely 5-6. Changes in pH affect the stability of phenolic compounds and flavonoids; both compounds will be more stable at low pH, because they are in the form of cations. According to Srivasta and Gupta [10] flavonoid compounds are stable at pH 5 -7, at pH 8 they are degraded by 6% while at pH 3-5 the degradation is not significant; whereas according to Friedman and Jurgens [11], chlorogenic acid polyphenols are unstable at high pH and stable at acidic and hot pH. This is supported by the measurement of total phenolic and total flavonoids which was higher in yacon syrup with inhibitors with a lower pH than yacon syrup without inhibitors (Table 1).

The antioxidant activity was indicated by the percentage of inhibition and the IC50 value. The results of the inhibition ability test and the calculation of the IC50 value are shown in Table 2.
Table 2. Percentages of Yacon Syrup Inhibition and IC50

| Treatments   | Syrup Concentration (% volume) | % Inhibition  | Average of % Inhibition | IC50 Value |
|--------------|-------------------------------|---------------|-------------------------|------------|
| (-) Inhibitor| 5                             | 14.905        | 24.388                  | 11.180     |
|              | 6                             | 18.723        |                         |            |
|              | 7                             | 24.151        |                         |            |
|              | 8                             | 29.208        |                         |            |
|              | 9                             | 34.954        |                         |            |
| (+) Inhibitor| 5                             | 47.637        | 52.888                  | 6.383      |
|              | 6                             | 50.760        |                         |            |
|              | 7                             | 52.823        |                         |            |
|              | 8                             | 55.151        |                         |            |
|              | 9                             | 58.068        |                         |            |

Syrup with inhibitor has a value of IC50 = 6.383, whereas without inhibitor IC50 = 11.180; The mean inhibition at a concentration of 5-9% by volume for the inhibited syrup and without the inhibitor was 52.888 and 24.388%. The IC50 value is the effective concentration of the extract needed to reduce 50% of the total DPPH. The IC value of 50 indicates that the syrup with an inhibitor is able to oxidize DPPH free radicals stronger than the syrup without an inhibitor, or it can be stated that inhibitor syrup has stronger antioxidant activity than syrup without inhibitor; the use of natural inhibitors can enhance the properties of antioxidant activity. This is due to the content of phenolic compounds as hydrogen atom donors to DPPH which support the antioxidant activity of the syrup. DPPH is a free radical compound capable of reacting with compounds that can donate hydrogen atoms.

Yacon-inhibited syrup has higher levels of phenolic compounds and flavonoids than without inhibitors; it will show better DPPH radical inhibitory activity or higher antioxidant activity. Testing through the DPPH method shows that the use of natural inhibitors can increase their antioxidant activity. This difference can be illustrated as in Figure 1.
The presumed antioxidant mechanism of chlorogenic acid against DPPH radicals is shown in Figure 2.

![Diagram of antioxidant mechanism of chlorogenic acid against DPPH radicals]

Figure 2. The Mechanism of DPPH Radical Inhibition by Chlorogenic Acid [12]

The process of scavenging free radicals is through the mechanism of taking hydrogen atoms from antioxidant compounds by free radicals so that free radicals capture an electron from the antioxidants. The synthetic free radicals used are DPPH. DPPH compounds react with antioxidant compounds by taking hydrogen atoms from antioxidant compounds to get electron pairs. Compounds that react as free radical scavengers will reduce DPPH which can be observed with the DPPH color change from purple to yellow when an odd electron from free radicals will form DPPH-H.

4. Conclusion
The findings showed that the use of natural inhibitors affects the antioxidant activity of yacon tuber syrup. The content of flavonoids and phenolic compounds in yacon syrup using natural inhibitors were 148.093±3.973 and 1244.595±67.672 ppm, significantly different from yacon syrup without inhibitors 100.923±9.277 and 978.912±54.223ppm. Antioxidant activity of syrup with natural inhibitors were 52.888% and IC50: 6.383, while yacon syrup without inhibitors were 24.388% and IC50: 11.180.

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