Production of purple sweet potato (Ipomoea batatas L.) juice having high anthocyanin content and antioxidant activity

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Abstract. The purpose of the study was to retrieve procedure of production of purple sweet potato (Ipomoea batatas L.) juice with the best total anthocyanin and antioxidant activity. Purple sweet potato was processed into purple sweet potato juice through a process of heating with temperature variations of 70°C, 80°C, and 90°C and various duration of heating, which are 5 mins, 10 mins, and 15 mins. The total anthocyanin was determined by using pH differential method. The antioxidant activity was determined by using DPPH (2,2-Diphenyl-1-picrylhydrazyl) method. Total anthocyanin of purple sweet potato juice declined in the range between 215.08 mg/L - 101.86 mg/L. The antioxidant activity of purple sweet potato juice declined in the range between 90.63% - 67.79%. Antioxidant activity and total anthocyanin purple sweet potato juice decreases with increasing temperature and duration of heating. The best characteristics found in purple sweet potato juice were made with warming temperatures of 80°C. The product with the highest antioxidant activity, total anthocyanins, and good durability was prepared at 80°C heating temperature for 5 mins.

1. Introduction
Food and beverages consumed by people can have two functions, first to meet the nutritional need and second to maintain the health and fitness of the body, improves physiological function or eliminate the negative effects of certain diseases. Food and beverages that have a second function is called functional foods and beverages.

Food and functional beverage contains natural substances called bioactive compounds. Bioactive compounds functioned as antioxidants which can react with free radicals and form a more stable free radical (not reactive). Antioxidants react with free radicals thereby reducing the capacity of free radicals to cause damage [1]. Therefore, antioxidants can prevent cell damage due to free radicals so that the body can be protected from the generative diseases such as coronary heart disease, cancer, diabetes, cataracts, and others.

Based on its source, antioxidants are grouped into synthetic antioxidants and natural antioxidants. The use of synthetic antioxidants is decreasing because it can cause negative health effects (liver damage) and may pose a carcinogenic substance, so its use is replaced by natural antioxidants [2]. The sources of natural antioxidants are fruits, vegetables, grains and tubers. One example of a natural source of antioxidants is purple sweet potato.
Purple sweet potato (*Ipomoea batatas*) skin and tuber flesh are dark purple-coloured. The purple sweet potato contains anthocyanin colour pigments. Total purple sweet potato anthocyanin content was 519 mg / 100 g fresh weight [3]. The purple pigment (anthocyanin) in purple sweet potato is useful as an antioxidant because it can react with free radicals in the body cells to reduce the capacity of free radicals that can cause damage in the body. According to [4], the pigment anthocyanin contained in purple sweet potato has a higher stability compared to other sources such as anthocyanin in red cabbage, elderberries, blueberries and red corn. The stability of the pigment anthocyanin is influenced by light, temperature, and pH [5].

Anthocyanins are sub-types of organic compounds of the flavonoid family and are members of a larger group of compounds called polyphenols. Some anthocyanin compounds include pelargonidin, peonidin, cyanidin, malvidine, petunidin, and delphinidin. The two anthocyanin components are cyanidin 3-O-(2-O-(6-O- (E)-caffeoyl)-D-glucopyranocyl-D-glucopyranoside)-5-OD-glucopyranoside and peonidin 3-O-(2-O-(6-O-(E)-caffeoyl)-D-glucopyranocyl-D-glucopyranoside)-5-OD-glucopyranoside contained in the purple sweet potato showed antioxidant activity [3].

Based on the results of previous researches, anthocyanins from purple sweet potato has the physiological functions as an active antioxidant and can reduce carbon tetrachloride-induced liver damage [5]. Eight major components of anthocyanins in purple sweet potato showed higher levels of antioxidant activity of ascorbic acid [6]. Other studies mentioned the purple sweet potato tuber extract can be used as an antioxidant in mice given a maximum load of physical activity [7].

Given the many benefits contained in purple sweet potato, innovation and creation are needed to process the purple sweet potato that has high antioxidant value. One way to process purple sweet potato is to produce purple sweet potato juice as functional beverage. One of the step of processing purple sweet potato into juice includes heating treatment. According to [8] the heating process can affect the stability of anthocyanin contained in purple sweet potato. Therefore, this study aims to determine the purple sweet potato processing which can produce purple sweet potato juice with the best antioxidant activity and levels of anthocyanin.

**2. Methods**

**2.1. Materials**

The main material used in this study was purple sweet potato. Other materials used in the process of making purple sweet potato juice were sugar and water. Materials to be used for testing was a pH indicator, KCl buffer pH 1.0, Na-Acetate buffer pH 4.5, methanol, DPPH (2,2-Diphenyl-1-picrylhydrazyl), and distilled water.

**2.2. Research Procedure**

**2.2.1. Preparation of Purple Sweet Potato Sample.** Purple sweet potatoes were sorted to choose good quality ones then washed with clean water. For the extraction process, the washed purple sweet potatoes were then mashed. As for the process of making the juice, purple sweet potatoes were steamed for 15 mins after water began to boil.

**2.2.2. Extraction of Purple Sweet Potato.** 100 grams of purple sweet potato was macerated with 200 mL of methanol for 1×24 hours and also macerated with 200 mL of aquades for another 1×24 hours. The obtained extract was then filtered and concentrated using a vacuum rotary evaporator.

**2.2.3. Making the Purple Sweet Potato Juice.** Steamed purple sweet potatoes were peeled and cut into small pieces then weighed as much as 100 grams and added with 200 ml aquades then blended until it becomes purple sweet potato porridge. The purple sweet potato porridge was filtered with a filter cloth and the filtrate was mixed with sugar. According to [9], the heating temperature in syrup was 85°C with prolonged heating 10 mins, so in this study were performed a variation of heating at
temperature of 70°C, 80°C, and 90°C and variations in the length of heating duration for 5, 10, and 15 mins. Purple sweet potato juice that has been obtained was added to the measuring flask of 250 mL and added with distilled water until it reached the marking on the flask, then put into bottles and pasteurized at a temperature of 75 °C for 10 mins, then purple sweet potato juice was cooled rapidly.

2.2.4. **Tests of Total Anthocyanin Concentration.** Total anthocyanin concentration measurement was performed by the method according to the pH difference [10]. Two sample solutions were prepared; the first solution was for the solution of pH 1.0 using KCl-HCl buffer (0.025 M) and the second solution was for pH 4.5 using Na-Acetate-HCl buffer (0.4 M). 1 mL of purple sweet potato extract and 1 mL of purple sweet potato juice of various heating temperature were each taken and diluted using each buffer solution until a volume of 10 mL (diluting factor = 10). Absorbance measurement at a wavelength of 530 nm and 700 nm was done on diluted samples, and the total concentration was determined using Equation 1:

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\text{Total anthocyanin (mg/L)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times L} \\
A : (\text{Abs} 530 \text{ nm} - \text{Abs} 700 \text{ nm}) \text{ pH } 1 - (\text{Abs} 530 \text{ nm} - \text{Abs} 700 \text{ nm}) \text{ pH } 4.5 \\
\varepsilon : \text{The molar extinction coefficient} \\
\text{MW} : \text{Molecular weight (449.2)} \\
\text{DF} : \text{Dilution factor} \\
L : \text{Cuvette thickness (1 cm)}
\]

2.2.5. **Test of Antioxidant Activity.** Determination of antioxidant activity by modified DPPH method according to [11]. To test the antioxidant activity of purple sweet potato juice, 0.5 mL of purple sweet potato juice was added to 3 mL methanol and 0.3 mL DPPH 0.5 mM. The mixture was incubated for 100 min at room temperature then its absorbance was measured using a UV-Vis spectrophotometer at 517 nm wavelength. The blank for measurement was made by mixing 3.3 mL methanol with 0.5 mL of sample. As for the control was made by mixing 3.5 mL methanol with 0.3 mL DPPH 0.5 mM. Antioxidant activity can be determined by Equation 2.

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\% \text{Antioxidant activity} = 100 - \frac{(\text{Sample absorb.} - \text{Blanko absorb.}) \times 100}{\text{Control absorb.}}
\]

2.2.6. **Test for Characteristic of Purple Sweet Potato Juice in Storage**

Purple sweet potato juice was tested for characteristics during storage at room temperature in a conventional manner through the observation of the physical condition and the measurement of pH. The test was done on purple sweet potato juice which are made on the heating temperature of 70°C, 80°C, and 90°C and heating duration of 5, 10, 15 mins. A total of 10 mL of purple sweet potato juice was put into each of the test tubes and stored at room temperature. Changes in physical conditions was observed and also measurement of pH was done every day until there was a change on the sample. This characteristics test is done for 5 days.

3. **Results and Discussion**

3.1. **Extraction of Purple Sweet Potato**

Purple sweet potato extract was obtained by immersing the sample in a suitable solvent [8]. This maceration process does not require heating so it will not damage the secondary metabolite compounds contained in purple sweet potato. Extraction of purple sweet potato was done by using two solvents, which were water and methanol solvent. Water was used as a solvent in the extraction process because water was one of the ingredients used in the manufacture of purple sweet potato
juice products. Methanol was used as a solvent because it is an effective solvent that can dissolve almost all of secondary metabolites present in the extracted sample of purple sweet potato. Methanol is also a solvent that has a low boiling point and is volatile that can be easily separated from the extract.

The methanol extracts obtained from the filtration was then evaporated using a vacuum rotary evaporator at a temperature of 46°C. From the evaporation obtained concentrated dark purple-coloured extract with volume 27 mL from initial volume of liquid extract of 179 mL.

Water extract obtained from the filtration was evaporated using a rotary vacuum evaporator with temperature of 68°C. From the evaporation obtained a red-coloured concentrated extract with a volume of 12 mL from initial volume of 176 mL.

3.2. Products of Purple Sweet Potato Juice
Purple sweet potato processed into juice by using two variables; heating temperature (70⁰C, 80⁰C, and 90⁰C) and heating time (5, 10, and 15 mins). At the beginning of the procedure, purple sweet potato samples were steamed for 15 mins to deactivate enzymes that might damage the quality of the juice. From the sample preparation obtained deep purple-coloured filtrate and light purple residue. Then added 10 grams of sugar to the extract and heated according to the predetermined temperature and heating variables. The results obtained were concentrated purple sweet potato juice.

The higher the heating temperature and the longer the heating time produces purple sweet potato juice with denser colors. The heating process will cause degradation of anthocyanin compounds into ketones brown product. The brown color of this ketone product cause purple sweet potato juice color becomes more concentrated.

3.3. Total Anthocyanin of Purple Sweet Potato Extract and Juice using Method of pH Difference
Purple colour of purple sweet potato indicated the presence of a natural dye contained in it which is anthocyanin, a water-soluble pigment and is a secondary metabolite compound of the flavonoid group that can act as an antioxidant in purple sweet potato. The total anthocyanin contained in purple sweet potato before and after processed into purple sweet potato juice was measured.

Measurement of total anthocyanin extracts and juices of purple sweet potato was done by using the method of pH difference. At pH 1 anthocyanin formed red oxonium compound (cation flavilium) and at pH 4.5 anthocyanin formed carbinol/colorless hemiketal [8]. This condition was used as a reference to determine absorbance by using UV-Vis spectrophotometer from each purple sweet potato extract and juice. The discoloration of anthocyanins in certain pH levels was due to the anthocyanin properties that have different stability levels. At pH 1 anthocyanin was more stable and the color was darker than pH 4.5 which was less stable and almost colorless. In addition to the pH factor, the presence of mixtures with other compounds (copigmentation), pigment concentrations, the number of hydroxyl groups and methoxy also affected the color of the anthocyanins. Absorbance of anthocyanin dissolved in buffer at pH 1 and pH 4.5 was measured at a wavelength of 530 nm which is the wavelength maximum of anthocyanin, while absorption at a wavelength of 700 nm performed as a correction factor. The measurement of total anthocyanin in extract of purple sweet potato can be seen in Table 1.

| Sample          | Total Anthocyanin (mg/L) |
|-----------------|--------------------------|
| Methanol extract| 361.69 mg/L              |
| Water extract   | 253.15 mg/L              |

On Table 1, it is shown that total anthocyanin in methanol extract of purple sweet potato is 361.69 mg/L greater than the total anthocyanin in water extract which is 253.15 mg/L. This indicates that methanol was better than water in terms of dissolving anthocyanin. The measurement results of total anthocyanin in purple sweet potato juice can be seen in Table 2.
Table 2. Results of Total Anthocyanin in Purple Sweet Potato Juice

| Time  | Heating Temperature |
|-------|---------------------|
|       | 70°C | 80°C | 90°C |
| 5 mins | 215.08 mg/L | 181.01 mg/L | 137.93 mg/L |
| 10 mins | 203.55 mg/L | 175.00 mg/L | 114.88 mg/L |
| 15 mins | 186.19 mg/L | 148.28 mg/L | 101.86 mg/L |

In Table 2, it can be seen that the higher the heating temperature and the longer the heating can reduce the total anthocyanins in purple sweet potato juice. Purple sweet potato juice made on the heating temperature 70°C within 5 mins have the best amount of total anthocyanin of 215.08 mg/L.

From the total anthocyanin data it can be seen that anthocyanin is unstable and susceptible to heating process, this is indicated by the increase of heating temperature and the heating duration, the total measured anthocyanin is lower due to degradation of anthocyanin. Anthocyanin degradation can cause the structure changes to ketone products. The formation of ketone products cause a reduction in the number of phenolic hydroxyl group of anthocyanins which act as hydrogen donors to free radicals thereby reducing its ability to reduce free radicals.

3.4. Antioxidant Activities of Purple Sweet Potato Extract and Juice using DPPH Method

The antioxidant activity of purple sweet potato extract and juice was determined using DPPH method. The principle of the DPPH method is the capture of hydrogen atoms by DPPH free radicals from antioxidants. DPPH method is chosen because it is an easy method, simple, and uses only a few number of samples with short processing time. In addition, DPPH is a stable radical so its use in determining antioxidant activity is much easier. Measurement of antioxidant activity was performed by using visible spectrophotometer at 517 nm wavelength.

The antioxidants contained in purple sweet potato extract and juice resulted in the colour change of DPPH solution. DPPH solution changes from purple to yellow. This occurred because the DPPH radical reacts with the hydrogen atoms donated by the antioxidant compounds in the extract and juice, resulting in reduced DPPH. The stronger the antioxidant compounds in donating a hydrogen atom, the higher the antioxidant activity is. The measurement results of the antioxidant activity of the purple sweet potato extract can be seen in Table 3.

Table 3. Results of Antioxidant Activity in Purple Sweet Potato Extract

| Sample         | Antioxidant Activity (%) |
|----------------|--------------------------|
| Methanol extract | 97.92%                   |
| Water extract   | 94.23%                   |

Results in Table 3 show that the antioxidant activity in methanol extract of purple sweet potato with 97.92% was greater than in water extract, 94.23%. This shows that methanol can dissolve the group of secondary metabolites that can act as antioxidants better. The measurement results of the antioxidant activity of the purple sweet potato juice can be seen in Table 4.

Table 4. Results of Antioxidant Activity in Purple Sweet Potato Juice

| Time  | Heating Temperature |
|-------|---------------------|
|       | 70°C | 80°C | 90°C |
| 5 mins | 90.63% | 83.68% | 80.72% |
| 10 mins | 87.65% | 82.41% | 77.54% |
| 15 mins | 86.17% | 81.35% | 67.79% |
In table 4 can be seen that the antioxidant activity decreases with rising temperatures and prolonged heating. Purple sweet potato juice made on the heating temperature 70°C within 5 mins have the best antioxidant activity of 90.63%.

Data of antioxidant activity above indicates that antioxidant is susceptible to heating process. The destruction of antioxidant compounds affect the purple sweet potato antioxidant activity after being processed into a purple sweet potato juice. This is seen in the decrease of antioxidant activity of purple sweet potato juice. The increasing temperature of heating and the duration of the heating of purple sweet potato juice resulting in lower antioxidant activity. One of the secondary metabolite compounds in purple sweet potato that can act as an antioxidant is anthocyanin. Anthocyanins are particularly susceptible to heating. Storage temperature and high temperature treatment process will cause degradation of anthocyanin compounds [8]. If anthocyanin is degraded, it can cause changes in the structure of anthocyanins into ketone product which can reduce its ability to reduce free radicals so that the antioxidant activity also decreases.

3.5. Characteristic of Purple Sweet Potato Juice in Storage

Testing characteristics of purple sweet potato juice during storage at room temperature was done through observation of physical condition and pH measurements on each purple sweet potato juice drinks which were made with a variety of heating temperature and heating times. The purpose of testing the characteristic of purple sweet potato drink was to determine which of the purple sweet potato juice that had the best shelf life. The purple sweet potato extract was stored at room temperature and the observation was done until the physical condition and pH changes. The test was done for 5 days.

The observation results of the physical condition and pH measurements are shown in Table 5.

| Day | Temperature and Time of Heating |
|-----|--------------------------------|
| 0   | 70°C (5, 10, 15 min.) | 80°C (5, 10, 15 min.) | 90°C (5, 10, 15 min.) |
|     | Dark purple-coloured juice with pH 6 | Dark purple-coloured juice with pH 6 | Dark purple-coloured juice with pH 6 |
|     | Fading colour, foam and precipitate formed, pH measured at 5 | Slightly fading colour with pH 6 | Dark purple-coloured juice with pH 6 |
|     | More colour fading, foam increases, smells acidic, pH measured at 5 | Colour fading, foam and precipitate formed, pH measured at 5 | Colour fading, precipitate started to form, pH measured at 5 |
|     | Colour turned to red, foam increases, pH measured at 4 | Colour turned to red, foam increases, pH measured at 5 | More colour fading, foam formed, pH measured at 5 |
|     | Colour gets redder, a white layer formed on the surface, pH measured at 4 | Red-coloured, foam and precipitate increase, pH measured at 4 | Colour turned to red, foam and precipitate increase, pH measured at 4 |

From the observation of the physical condition of the sample, it is known that the heating temperature can inhibit microbial growth. It is seen in the higher temperature of heating, then the
microbial growth was more hampered so that the physical condition of purple sweet potato juice can be more preserved.

Based on the results of measurements of the antioxidant activity and total anthocyanin purple sweet potato juice that have the highest antioxidant activity and total anthocyanin is the one made with heating temperature of 70°C for 5 mins, resulting in antioxidant activity of 90.63% and total anthocyanin of 215.08 mg/L. But on the test results of characteristics of purple sweet potato juice during storage which is done through observation of the physical condition and pH measurements showed that the purple sweet potato juice made with heating temperature of 70°C for 5 mins shows changes in the physical conditions which are colour fading and forming of foam and precipitation within only 1 day. Based on the results of characteristics test, purple sweet potato juice beverage that has the best shelf life is the purple sweet potato juice made with heating temperature of 80°C. Thus, the purple sweet potato juice that have quite high antioxidant activity and total anthocyanin and has a durable shelf life for 2 days is the purple sweet potato juice drinks which is made with heating temperature of 80°C for 5 mins, with antioxidant activity of 83.68% and total anthocyanin of 181.01 mg/L.

4. Conclusions
Based on the results of research and discussion explained above, conclusions reached are the antioxidant activity and total anthocyanin in purple sweet potato juice decreases with rising temperatures and prolonged heating and the procedures for making juice purple sweet potato that produces antioxidant activity, total anthocyanins, and a fairly good shelf life are with heating temperature of 80°C for 5 mins.

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