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Research Article

Analysis of antioxidant activity of carrots (*Daucus carrota* L.) using FRAP and CUPRAC methods

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ABSTRACT

Carrot antioxidant activity analysis has been carried out. Carrot juice was analyzed qualitatively by the FRAP method using FRAP reagent and by the CUPRAC method using copper (II) chloride solution, neucuproine solution, ammonium acetate buffer solution, and distilled water. Quantitatively, the mixture of sample solutions using the FRAP and CUPRAC methods was analyzed using a UV-Vis spectrophotometer at a wavelength of 596 nm for the FRAP method and 452 nm for the CUPRAC method, using Trolox solution as the standard curve. The results obtained were that each sample A and B's antioxidant activity using the FRAP method was 0.5186 µM TR/g and 0.8857 µM TR/g samples. Meanwhile, samples A and B's antioxidant activity using the CUPRAC method was 0.1160 µM TR/g sample and 0.1762 µM TR/g sample.

1. INTRODUCTION

Free radicals are compounds or molecules that contain one or more unpaired electrons in their outer orbitals. The presence of unpaired electrons causes the compound to be very reactive, looking for a partner by attacking and binding the electrons of the molecules around it (Winarsi, 2007). Free radicals will react with surrounding molecules to obtain electron pairs to become stable, but the body molecules that have taken electrons then turn into free radicals. This reaction will occur continuously in the body, and if not stopped, will cause cell damage and various diseases such as cancer, heart disease, cataracts, and other degenerative diseases. Therefore, the body needs a vital substance, namely antioxidants, that can capture these free radicals so that the radical compounds become stable and cannot induce a disease (Halliwell & Gutteridge, 2000).

Antioxidants are compounds that give electrons (electron donors). This compound has a small molecular weight but can inactivate the development of oxidation reactions by preventing radicals' formation. Antioxidants are also compounds that can inhibit oxidation reactions by binding to free radicals and highly reactive molecules. As a result, cell damage will be inhibited it (Winarsi, 2007). The mechanism of antioxidants in inhibiting oxidation or stopping the chain reaction of free radicals from oxidized fats can be caused by four reaction mechanisms, that is release of hydrogen from antioxidants, release of electrons from antioxidants, addition of fats into the aromatic ring on antioxidants, and formation of complex compounds between fats and the aromatic ring of antioxidants (Ketaren, 2008).

Carrots (*Daucus carrota* L.) are a type of vegetable widely consumed by Indonesians as a cooking ingredient. Carrots are also vegetables that can be natural antioxidants, where carrots themselves contain β-

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carotene, which acts as a natural antioxidant. Momuat, Sangi and Purwati (2010), suggests that β-carotene is an orange pigment found in carrots and islipid-soluble, and has a role as an antioxidant. Carotenoids' ability as antioxidants lie in the chemical structure of β-carotene, which contains several conjugated dienes that cause oxidation reactions (Huang, Ou & Prior, 2005).

Based on this, a study was conducted to measure and determine the antioxidant activity of carrots using the FRAP (Ferry Reducing Antioxidant Power) (Benzie & Strain, 1996) and CUPRAC (Cupric Ion Reducing Antioxidant Capacity) methods (Özyürek et al., 2011). Researchers hope that the results of this research will be useful for the development of science.

2. MATERIALS AND METHODS

This research was conducted to analyze the antioxidant activity of carrots with the FRAP and CUPRAC methods, where these two methods are methods used for the analysis of antioxidant activity. This type of research is an experimental laboratory type. This study also used two different types of carrots obtained from supermarkets.

This research was conducted at the Chemical Laboratory of the Muslim University of Indonesia. Samples analyzed using the FRAP method was proceeded as follows: carrots were cleaned and then crushed in a juicer; then 5 mL of samples were taken and centrifuged for 10 minutes at 3000 rpm, after which 1 mL of supernatant is taken and diluted with 5 mL of ethanol then it will be mixed with 3 mL of FRAP reagent after that the mixture is incubated at 37 °C for 30 minutes. The absorbance is measured at λ_{max} 596 nm with a UV-Vis spectrophotometer. The Trolox is done by making a stock solution 600 µM made by dissolving 15 mg trolox dissolved in ethanol for the comparison used and then diluted to the limit of 100 mL volumetric flask. Furthermore, from a stock solution of 600 µM, the volume of 0.83; 1.6; 2.5; 3.3; 4.1; and 5 mL were placed in a different volumetric flask and diluted with ethanol to 5 mL and then homogenized. The standard Trolox solution concentrations were 100; 200; 300; 400; 500; and 600 µM, respectively. The same thing was done as the sample with the addition of reagents used in the FRAP method.

As for the sample using the CUPRAC method, the carrot samples were prepared as follows: the carrots were cleaned first, then weighed as much as 100 gr, crushed in the juicer, and added with 1 mL trichloroacetic acid. The liquids were centrifuge for 10 minutes at 3000 rpm. Then 2 mL of supernatant was taken and diluted with 5 mL ethanol, then 0.5 mL was taken then mixed with 1 mL of copper (II) chloride solution, 1 mL of neocuproine solution, 1 mL of ammonium acetate buffer, and 0.6 mL of distilled water (total volume = 4.1 mL), the mixture was incubated at 37 °C for 30 minutes. The absorbance is measured at λ_{max} 452 nm with a UV-Vis spectrophotometer. The Trolox is done by making the workstock solution 600 µM made by dissolving 5 mg Trolox dissolved in ethanol for the comparison used and then diluted to the limit of 100 mL volumetric flask.

Furthermore, from the 600 µM stock solution, 0.3; 0.6; 0.8; 1.6; and 3.3 mL respectively placed in a different volumetric flask and diluted with ethanol to 5 mL and homogenized. The standard Trolox solution concentrations were 40; 80; 100; 200; and 400 µM, respectively. The same thing was done as the sample with the addition of the reagent used in the CUPRAC method.

3. RESULTS AND DISCUSSIONS

Carrot is a plant that is a genus of the family Umbelliferae. Carrot is one type of tuber vegetable that has a vital role in providing food, especially the supply of vitamins and minerals. Carrots contain lots of vitamin A and other substances with medicinal properties, perfect for preventing various diseases. Carrots also have good taste (slightly sweet), so they are very popular with people. Based on the chemical content of carrots including vitamin C and β-carotene , which are compounds that have potential as antioxidants, an analysis of the antioxidant activity of this plant was carried out using FRAP and CUPRAC methods.

FRAP method is a method used to test antioxidants in plants. The advantages of this FRAP method are that it is cheap, fast, and the reagents used are quite simple and do not use special tools to calculate total antioxidants (Szőllősi & Varga, 2002). Determination of the value of antioxidant activity was carried out by mixing the FRAP reagent with carrot samples. Where in the FRAP reagent, there is a mixture of TPTZ, $FeCl_3$, and acetate buffer. The addition of $FeCl_3$ in the reagent is to form Fe^{3+} complex compounds and slow down the Fe^{3+} reduction reaction to Fe^{2+}, which occurs very quickly under the influence of light. At the same time, the addition

Table 1. The results of measuring the absorbance and antioxidant activity value of carrots using the FRAP method

Carrot	Replication	Absorbance (596 nm)	Antioxidant activity (µM TR/g sample)	Average (µM TR/g sample)
A	I	0.352	0.5175	0.51860
	II	0.340	0.5044	
	III	0.367	0.5339	
B	I	0.713	0.8833	0.88570
	II	0.698	0.8674	
	III	0.735	0.9066	

Table 2. The results of measuring the absorbance and antioxidant activity value of carrots using the CUPRAC method

Carrot	Replication	Absorbance (596 nm)	Antioxidant activity (µM TR/g sample)	Average (µM TR/g sample)
A	I	0.200	0.1250	0.1160
	II	0.221	0.1364	
	III	0.130	0.0860	
B	I	0.292	0.1697	0.1762
	II	0.299	0.1734	
	III	0.322	0.1856	

of acetate buffer is because this buffer has an effective pH of 3.6 - 5.6 (Gandjar & Rohman, 2007). Where it is known that the reduction reaction of Fe^{3+}-TPTZ to Fe^{2+}-TPTZ occurs in an acidic atmosphere or low pH, so acetate buffer is used with the lowest effective pH of 3.6. Ou, Huang, Hampsch-Woodill, Flanagan and Deemer (2002) states that Fe^{3+}-TPTZ represent oxidizing compounds that may be present in the body and damage body cells, while samples contain antioxidants that can then reduce Fe^{3+}-TPTZ to Fe^{2+}-TPTZ, so that Fe^{3+}-TPTZ will not perform reactions that damage body cells. The more the concentration of Fe^{3+}-TPTZ, which is reduced by the sample to Fe^{2+}-TPTZ, the greater the antioxidant activity of the sample. This complex is stable at acidic pH, so pH 3.6 was used in this study. The use of low pH is intended to facilitate the Fe^{3+} reduction process.

The formation of a blue color causes an increase in the absorbance value of the sample. The more blue the color formed in the sample, the higher the absorbance value. This result showed the antioxidant activity contained in the carrot plant. Samples with high reducing power are excellent electron donors who can stop radical chain reactions by converting free radicals into more stable products. The antioxidant activity of reductants is based on the breakdown of radical chains due to hydrogen atoms' addition.

The concentration (x) regression results with the absorbance value (y) of the standard Trolox then obtained the equation, namely y = 0.001x - 0.122 with a value of R^2 = 0.991 absorbance of the sample was entered into the equation. For sample carrot A, the first replication has absorbance 0.352 with antioxidant activity 0.5175 µM TR/g, the second replication has absorbance 0.340 with antioxidant activity 0.5044 µM TR/g, and the third replication has absorbance 0.367 with antioxidant activity 0.5339 µM TR/g. The average value of the sample carrots A was 0.5186 µM TR/g. For sample carrot B, the first replication has absorbance 0.713 with antioxidant activity 0.8833 µM TR/g, the second replication has absorbance 0.698 with antioxidant activity 0.8674 µM TR/g, and the third replication has absorbance 0.735 with antioxidant activity 0.9066 µM TR/g. The average value of the sample carrots B was 0.8857 µM TR/g.

While the CUPRAC method has advantages such as stable reagent, easy to obtain, works at physiological pH, and can measure hydrophilic and lipophilic antioxidants (unlike DPPH, which is only able to detect alcohol-soluble compounds). In addition, the CUPRAC redox reaction to various flavonoids does not take long. Selective oxidation of antioxidant compounds is not affected by the content of citric acid and sugar in foodstuffs. It can test antioxidants that have thiol bonds (Apak et al., 2007). Bis(neocuproine)Cu(II) chelate reagent was used as a chromogenic oxidizing agent because the reduction of Cu(II) ions could be measured. The absorption formed by Cu(I)-$(Nc)_2$ chelate as a result of reduction by antioxidant compounds was measured at a wavelength of 450 nm. The reactions that occur between Cu(II)-$(Nc)_2$ reagents with antioxidant compounds are

$$n\ Cu(Nc)_2^{2+} + n\text{-electron reductant (AO)} \leftrightarrow nCu(Nc)^{2+} + n\text{-electron oxidation product} + n\ H^+ \quad (1)$$

The color change when adding solvent that can be seen visually follows the theory, which states visually that the color change can be seen from the change in the color of the solution complex (Apak et al., 2007).

The concentration (x) regression results with the absorbance value (y) of the standard Trolox then

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obtained the equation, namely y = 0.001x- 0.029 with a value of R^2 = 0.979 absorbance of the sample was entered into the equation. For sample carrot A, the first replication has absorbance 0.200 with antioxidant activity 0.1250 µM TR/g, the second replication has absorbance 0.221 with antioxidant activity 0.1364 µM TR/g, and the third replication has absorbance 0.130 with antioxidant activity 0.0868 µM TR/g. The average value of the sample carrots A was 0.1160 µM TR/g. For sample carrot B, the first replication has absorbance 0.292 with antioxidant activity 0.1697 µM TR/g, the second replication has absorbance 0.299 with antioxidant activity 0.1734 µM TR/g, and the third replication has absorbance 0.322 with antioxidant activity 0.1856 µM TR/g. The average value of the sample carrots B was 0.1762 µM TR/g.

In this study, the comparison of Trolox was used because it is a synthetic antioxidant. The Trolox structure is similar to α-tocopherol and has a higher antioxidant activity than α-tocopherol, BHA, and BHT (Belitz et al., 2009). Trolox is often used as a standard in measuring antioxidants. The TEAC (Trolox equivalent antioxidant capacity) coefficient is the Trolox concentration with an antioxidant capacity equivalent to the analyzed sample. Each method's antioxidant capacity is expressed in µM TR/g (Widyastuti, 2010).

From these data, it can be seen that the difference in the value of antioxidant activity indicates that the value of antioxidant activity in the sample carrot B is greater than the value of the antioxidant activity in the sample carrot A. According to Cahyono (2002), these differences can be influenced by the method of harvesting, the way of maintaining the plants, the place where they are grown, the soil conditions, and climatic conditions such as temperature, rainfall, humidity, sunlight and wind. As for the method used, the FRAP method showed that the antioxidant activity measured was more significant than the CUPRAC method.

4. CONCLUSIONS

From the research results, it can be concluded that the carrot sample used has antioxidant activity in both methods, the antioxidant activity value of sample B is more significant than sample A. The method used shows that the antioxidant activity of the sample measured by the FRAP method is greater than the CUPRAC method.

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