

Antioxidant activity and total weight of carotenoids in red sweet potato (*Ipomoea batatas* L.) tuber

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ABSTRACT

West Java is the 2nd highest after Papua in the harvested of sweet potato. Sweet potato is a food alternative after corn. The nutritional content of sweet potato lower than rice, but sweet potatoes has phytochemicals content which has health benefits. One of its phytochemical is carotenoids which act as an antioxidant nutrient. The objective of the research is to evaluate antioxidant activity and the total weight of carotenoids of local sweet potato. Carotenoids were isolated by column chromatography method with alumunium oxide as stationer phase and n-hexane as mobile phase. Carotenoids present were identified with measure of its ultraviolet spectrum, total weight was weighed with a balance, and antioxidant activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Spectrum of carotenoids have two peaks were 449 and 475 nm. In fresh sample total weight of carotenoids was 62.1386 mg/100g, and antioxidant activity was 4.89 mg/ml. In conclusion, the local sweet potato harvested from Maja (West Java) contains carotenoids which effectively reduce blindness and eye diseases, related to β -carotene. Besides that, it also contains antioxidant which helps in preventing diseases caused by free radicals.

Keywords: Antioxidant activity, carotenoids, DPPH, *Ipomoea batatas*, sweet potato

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INTRODUCTION

Sweet potatoes had an important role as food, as sources of: carbohydrates, an energy and phytochemicals in human nutrition, one of the content of red sweet potato is carotenoids. Carotenoids are: α -carotene, β -carotene, γ -carotene, ϵ -carotene and lycopene. The major function of beta-carotene is as provitamin A. It can act as a lipid radical scavenger and as a singlet oxygen quencher (SEC, 2010, Pochapski et al., 2010, Panda et al., 2012, Grune et al., 2010).

Function of carotenoids in human health was published: they are recognized as playing an important role in the prevention of human diseases and maintaining good health. Carotenoids have been considered to provide benefits in age-related diseases, against some forms of cancer (in especial lung cancer), strokes, macular degeneration, and cataracts (Panda et al., 2012, Rao et al., 2007, Padgaonkar et al., 2008, Kadian et al., 2012).

The other phytochemical in tuber were sesquiterpenoids which include 6-myiporol, 4-hydroxy-dehydromyoporone and ipomeamarone, two storage proteins: saponin 1 and 2, account for more than 89% of the total proteins (Panda et al., 2012).

As herbal medicine the antioxidant capacity of sweet potato was 42.94% as compared to ascorbic acid (Pochapski et al., 2010). The aqueous whole plant extract of *Ipomoea batatas* were tested of their effects on the fasting blood glucose of normal and STZ-induced hyperglycemic rats. The doses 100, 200 and 400 mg/kg/day were administered as single, daily oral treatment for 14 days, and showed their effects on the fasting blood glucose caused significant dose related reductions ($P < 0.05$, $P < 0.001$) (Olowu et al., 2011). Anticancer activity of sweet potato greens in prostate cancer was published that oral administration of 400 mg/kg of sweet potato green extract remarkably inhibited growth and progression of prostate tumor xenografts by ~69% in nude mice, as shown by tumor volume measurements and non-invasive real-time bioluminescent imaging. Most importantly, of sweet potato green extract did not cause any detectable toxicity to rapidly dividing normal tissues such as gut and bone-marrow. (Karna et al., 2011).

Some foods have the ability to help stave off cancer and some can even help inhibit cancer cell growth or reduce tumor size. Avocado, broccoli, carrots, chili peppers, sweet potato, etc. Sweet potato contain many anticancer properties, including beta-carotene, which may protect DNA in the cell nucleus from cancer-causing chemicals outside the nuclear membrane (The Cancer Cure Foundation, 2010).

Beta-carotene can isolation with column chromatography used alumina as stationer phase and hexane as mobile phase (Williamson, 1999). The spectra of carotenoids are quite characteristic between 400 – 500 nm, with a major peak around 450 nm and usually two minor peaks either side. The maximum spectra (nm) in petroleum ether or n-hexane are: α -carotene: 422, 444, 473; β -carotene: 425⁺, 451, 482; γ -carotene: 437, 462, 494; ϵ -carotene: 419, 444, 475, and lycopene: 446, 472, 505 (Harborne, 1984).

West Java is the 2nd highest after Papua in the harvested of sweet potato, boiled sweet potato tuber is commonly sold with bajigur drink (a traditional hot drink of Sundanese people) on the street by hawkers. It is a snack for porter, street sweeper, pedicab driver, etc. Beside cheaper it can give feel full and energy. Nowadays it made modern snacks such as: donate, chip, starch, flour, etc (SEC, 2010). Therefore the evaluation of antioxidant activity to against free radicals and total carotenoid to maintaining good health is important.

METHOD

Material:

Red sweet potato cultivated at Maja (Majalengka Regency), was harvested in March, 2013. It has length 12 – 15 cm; diameter 5 – 6 cm.

Reagent : DPPH (Sigma Aldrich), n-hexane (E.Merck).

Aluminiumoxid 60 G neutral (Typ E), (E.Merck)

Equipment:

Spectrophotometer : Eppendorf Biospectrometer Basic AG 22331 Hamburg seri:6135BJ

(German)

Spectrophotometer Hewlett Packard 8453 (USA)

Balance: Sartorius 2442 (USA)

Isolation and calculation the total weight of carotenoid, and preparation for column chromatography (CC).

Fresh sweet potato was blended. 10 gram was weigh and was dried on water bath.

Carotenoids was extracted from dried sweet potato with n-hexane, repeat the extraction until n-hexane clear (no color).

Collect all solution of carotenoid in n-hexane together and then n-hexane was evaporated.

Then dried carotenoids was prepared for was purified by column chromatography, it mixed with Aluminiumoxid 60 G neutral.

Column chromatography (CC)

The crude carotenoid is to be chromatographed on an 8 cm column of basic or neutral alumina, prepared with hexane as solvent. Run out excess solvent, and add the 200 mg of alumina that has the crude carotenoids absorbed on it. Add a few drops of hexane to wash down the inside of the CC and to consolidate the carotenoid mixture at the top of the column.

Elute the column with hexane, discard the initial colorless eluate, and collect all yellow or orange eluates together (Williamson KL, 1999).

Identification of carotenoids:

The spectrum of eluate was measure with a spectrophotometer Hewlett Packard 8453 (Harborne JB, 1984).

Calculation of total weight of carotenoid

The total weight was measured with a balance Sartorius 2442:

The eluate was dried.

The total weight of carotenoids was calculated with the equation below:

$$\text{The total weight of carotenoids} = \text{total weight of carotenoids with beaker glass} - \text{weight of beaker glass}$$

Antioxidant assay with DPPH:

Preparation of DPPH stock solution:

DPPH was prepared in methanol at concentration 1.0×10^{-3} M

The dried carotenoids was dissolved in methanol, and were made in 5 different concentrations: 4,65; 3,10; 1,55; 0,78; 0,39 mg/50 ml.

Assay of antioxidant activity with 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH):

Each of the samples was mixed with 1 ml DPPH stock solution, kept in dark for 30 min and optical density was measured at 517 nm using a spectrophotometer Eppendorf Biospectrometer Basic AG 22331. The absorbance was recorded. The result is % effective inhibition, and was calculated by the equation below:

$$\text{EC \%} = \frac{\text{reference absorbance} - \text{sample absorbance}}{\text{reference absorbance}} \times 100\%$$

RESULT AND DISCUSSION

Identification of carotenoid:

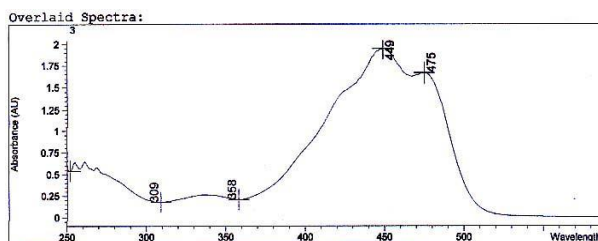


Figure1. Spectrum of carotenoids

The spectrum has two peaks at 449 and 475 nm and a shoulder. The spectra of carotenoids are quite characteristic between 400 – 500 nm. The ultraviolet spectra of each carotenoids in n-hexane are:

α -carotene: 422, 444, 473; β -carotene: 425[†], 451, 482; γ -carotene: 437, 462, 494; ϵ -carotene: 419, 444, 475, and lycopene: 446, 472, 505.⁵ Therefore the spectrum was suggested carotenoids.

Total weight of carotenoids in 9.9789 g fresh sweet potato = 12.9399 – 12.8778 g = 0.0062 g

$$\text{Total weight of carotenoids in 100 g fresh sweet potato} = \frac{100}{9.9789} \times 0.0062 \text{ g} = 0.0621 \text{ g}$$

$$= 62.1 \text{ mg}$$

Carotenoids is good for the prevention of blindness and eye disease, thus sweet potato has benefit to promote eye health.

Antioxidant activity of 6.2 mg carotenoids:

6.2 mg carotenoids was dissolved in 50 ml methanol, and were made in 5 different concentrations: 4,65; 3,10; 1,55; 0,78; 0,39 mg/50 ml.

Table 1. Percent inhibition of carotenoids on DPPH

No	Concentration of samples (mg/50 ml)	Absorbance of sampel							% inhibition
		1	2	3	4	5	6	Average	
1	4,65	0,981	0,954	0,955	0,969	0,977	0,971	0,967	7,01
2	3,10	0,952	0,956	0,953	0,955	0,971	0,973	0,960	7,69
3	1,55	0,932	0,943	0,951	0,951	0,966	0,969	0,952	8,46
4	0,78	0,991	0,985	1,002	0,999	0,959	0,964	0,983	5,48
5	0,39	0,944	0,952	0,982	0,981	0,979	0,979	0,969	6,83
Reference	1,040								

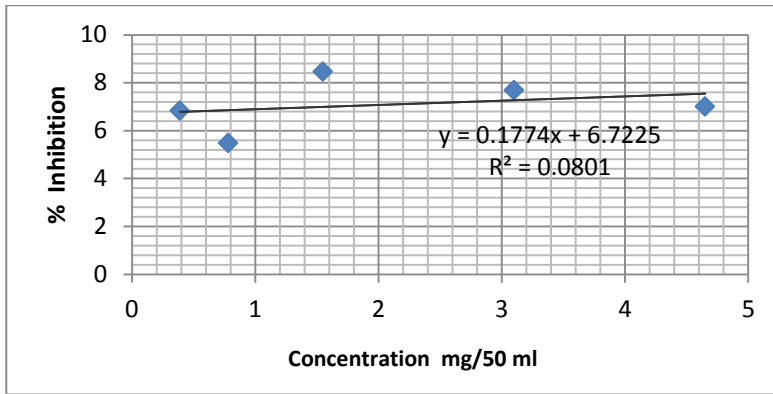


Figure 1: Percent inhibition of decoction of carotenoids on DPPH

EC 50% (x) =

$$y = 0,177x + 6,722$$

$$50 = 0,177x + 6,722$$

$$EC_{50} = (50 - 6,722) : 0,177 = 244,50 \text{ mg/50 ml} = 4.89 \text{ mg/ml}$$

Antioxidant activity E_{50} of fresh sweet potato was 4.89 mg/ml, thus sweet potato has activity to prevent the body from free radical which can cause diseases.

CONCLUSION

There, based on the study, the local sweet potato harvested from Maja (West Java) contains the total weight of carotenoids in 100 g fresh sweet potato was 62.1 mg which effectively reduce blindness and eye diseases, related to β -carotene. Besides that, the sweet potato also contains antioxidant with EC_{50} was 4.89 mg/ml which helps in preventing diseases caused by free radicals.

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