# Effect of Microwave Treatment on Antioxidant Activity of Anthocyanins in Purple Sweet Potato

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**ABSRACT----** ***In the present study, purple sweet potato was treated with microwave then measured the effect of microwave on antioxidant activity of anthocyanins from purple sweet potato. The objective of this study was to investigate the change of antioxidant activities of anthocyanins from purple sweet potato after treated with microwave in different analytical methods: the DPPH radical-scavenging effect, ABTS radical-scavenging effect, ferric reducing power, reducing power and superoxide anions radical-scavenging effect. The results displayed that microwave treatment greatly increased the antioxidant activity of anthocyanins extracted from purple sweet potato. The abilities of scavenging DPPH radical, ABTS radical and reducing power were much stronger than untreated samples. However, the ability of scavenging superoxide anions radical treated by microwave at 8 min was reduced. In conclusion, the microwave treatment could influence antioxidant activity of purple sweet potato, and the suitable microwave treatment could improve antioxidant activity.***

**Keywords----** Purple sweet potato; microwave treatment; antioxidant activity

## 1. INTRODUCTION

Purple Sweet Potato, belonging to Convolvulaceae, is an annual herb, originating in southern Kyushu of Japan. It was introduced to China in the 1990s and was successfully planted [1]. Purple sweet potato tuber is rich in minerals, dietary fiber and vitamins, especially rich in anthocyanins [2], with inhibition of cholesterol and cancer prevention, anti-cancer and other health care functions[3] .

Anthocyanins are water-soluble substances that are easily soluble in methanol, ethanol, water and mixed solutions composed of them. They are insoluble in non-polar solvents such as petroleum ether, and their color stability are affected by many factors such as structure, temperature, light, pH and other factors, and the impact of various factors vary greatly [4]. Studies have shown that anthocyanins have a strong role in the removal of oxygen free radicals, and delaying aging [5]. Purple sweet potato can be used to develop health food and functional foods, which is a kind of food materials of more and more people's favorite and concerns.

Microwave has penetration and selectivity, high heating efficiency. The pretreatment effect of microwave on antioxidant activities of anthocyanins in purple sweet potato have not been reported in the literature. In this experiment, purple sweet potato was pretreated by microwave for different time, and the changes of antioxidant activity after microwave treatment were analyzed in order to provide some theoretical guidance for the development of bioactive material of purple sweet potato.

## 2. MATERIAL AND METHODS

### *2.1 Raw materials pretreatment*

Fresh purple sweet potato, no pests, no mechanical damage, were chosen and washed from the sediment and other dirt with water. The washed purple sweet potato is cut into uniform slices of 0.3-0.5 cm and divided equally into three groups. The first group was treated with microwave power of 700 W for 4 min and the second group was treated with microwave of 700 W for 8 min. The third group served as a control without microwave treatment. Then three groups of purple sweet potato were completely dried into a blast oven at 60 ℃ temperature. The dried purple sweet potato chips crushing with high-speed multi-energy mill, the resulting purple sweet potato powder stored in the refrigerator at 4 °C.

### *2.2 Extraction of anthocyanin from purple sweet potato samples*

500 g of purple sweet potato powder under three different treatment were weighed. 95% ethanol as extractant, according to the ratio of solid to liquid 1:20 (g: mL), powder and extractant were mixed and putted in 60 °C water bath for extraction. The supernatant was evaporated on a rotary evaporator and finally the resulting concentrate was freeze-dried as powder for later use [6].

***2.3 DPPH free radical scavenging rate determination***

Different concentrations of extracts were prepared as a series of concentrations of the solution, respectively 0.1, 0.2, 0.3, 0.4, 0.5 mL sample solution and filled up to 0.5 mL with methanol. 3 mL 60 μmol / L of DPPH methanol solution was thoroughly mixed, sealed and reacted in Dark for 30 min at room temperature. Blank was replacing sample with methanol, absorbance measured at 517 nm [7].

DPPH free radical scavenging rate R is calculated as follows:

$$R=\left[1-\frac{A_1-A_2}{A_0}\right]\times 100\%$$

Where: $A_0$- absorbance value of tube with only DPPH;
$A_1$ - absorbance value of sample and DPPH;
$A_2$ - absorbance value of tube with sample and methanol.

***2.4 ABTS free radical scavenging rate determination***

$ABTS^+$ Radical Solution Preparation: ABTS solution with a concentration of 7 mmol / L and 2.45 mmol / L potassium persulfate solution were mixed. The mixture was placed in the dark at room temperature for 16-24 h, and then diluted with methanol to obtain application solution with the absorbance of 0.700 ± 0.050 at 734 nm.

Different concentrations of extracts formulated as a series of concentrations of the solution. 0.1 mL sample solution and 3.8 mL $ABTS^+$ free radical application solution were added, shacked and react in dark for 6 min at room temperature. The absorbance was measured at a wavelength of 734 nm immediately, the blank using methanol to replace sample [8].

ABTS radical scavenging rate R is calculated as follows:

$$R=\left[1-\frac{A_1}{A_0}\right]\times 100\%$$

Where: $A_0$- absorbance value of blank tube;
$A_1$ - absorbance value of sample tube.

***2.5 Determination of iron reducing ability (FRAP)***

0.1 mL of the pigment, 3.1 mL of distilled water and freshly prepared 1.8 mL of TPTZ working solution (300 mmol / L acetate buffer at pH 3.6, TPTZ solution of 10 mmol / L solution in 40 mM HCl, 20 mmol / L ferric chloride solution mixed by 10: 1: 1 volume ratio) were added in subsequence. The volume was fixed to 20 mL. After reaction for 30 min in 37 ℃ water bath, the absorbance was measured at 593 nm [9].

Using $FeSO_4$ as a standard, the regression equation was established. The iron reduction capacity of the sample is expressed in per millimoles gram of sample corresponding to $FeSO_4$.

Preparation of standard curve: A series of different concentrations of $FeSO_4$ were treated according to the above conditions, the absorbance was measured to obtain the concentration - absorbance curve, as shown:

***2.6 Determination of the scavenging rate of superoxide anion free radical (O2-)***

0.1 mL different concentrations of pigment, 9.0 mL 0.05 mol / L pH 8.0 PBS buffer was added and kept in 25 °C water bath for 15 min. Then taking 3.0 mL mixed solution in a test tube, 0.1 mL 45 mmol / L of pyrogallol were taken to react for 3 min. The absorbance value ($A_2$) at 420 nm was measured, and the absorbance value ($A_1$) of the sample before addition of pyrogallol was measured. The absorbance value $A_0$ of autoxidation of pyrogallol with 1.0 mL of absolute ethanol and $A_2$ of autoxidation of pyrogallol with sample were measured [10].

Superoxide anion free radical scavenging rate R is calculated as follows:

$$R=\left[1-\frac{A_{2-}A_1}{A_0}\right]\times 100\%$$

***2.7 Determination of reducing power***

2.5 mL 0.2 moL/L pH6.6 phosphate buffer solution, 2.5 mL 1% potassium ferricyanide solution and 0.1 mL sample solution of different concentrations were added sequentially in a 10 mL test tube, and the solution was putted in a 50 °C water bath for 20 min and then rapidly cooled down. Then 2.5 mL 10% trichloroacetic acid solution was add to stop the reaction and the mixture was centrifuged at 3000 r / min for 10 min. Taking 2.5 mL supernatant, 2.5 mL distilled water and 0.2 mL 0.1% ferric chloride solution were added, and stand for 10 min. Anhydrous ethanol as a reference solution, absorbance at 700 nm wavelength was measured [11].

## 3. RESULTS AND DISCUSSION

***3.1 Scavenging DPPH free radical assay results***

DPPH is a stable nitrogen center free radical whose stability mainly comes from the space obstacle of the three benzene rings that act as resonance stabilizers so that the unpaired electrons of the nitrogen atoms trapped in the middle

cannot exert their proper electron pair effect [12]. Due to the short reaction time and simple determination method, the scavenging ability of DPPH free radical has been widely used to evaluate the antioxidant capacity of fruits and vegetables and its extracts [13]. Antioxidants can react with the steady-state dark purple DPPH radical to convert to colorless 1,2-diphenyl-2-trinitrobenzene [14], which affects the absorbance value of the solution, and the higher the clearance rate, the stronger the antioxidant capacity.

As can be seen from Fig. 1, the scavenging DPPH activity of different concentrations of anthocyanins from purple sweet potato treated by microwave increased to different extent, and scavenging DPPH ability increased as the microwave treatment time increased. The scavenging activity of purple sweet potato treated by microwave for 8 min was significantly higher than the sample of 4 min and non-microwave treatment. As the sample concentration increased, the DPPH clearance rate of purple sweet potato treated by microwave for 4 min was higher than that of untreated sample. Therefore, appropriate microwave treatment can improve scavenging DPPH ability of purple sweet potato.

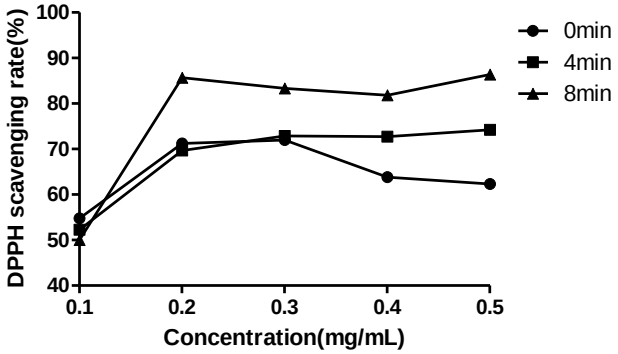


Fig1 scanvening DPPH activity of purple sweet potato processed by different microwave treatment

***3.2 Scavenging ABTS free radical assay results***

ABTS is fast and simple, and has strong correlation with the biological activity of antioxidants. It is widely used in the determination of antioxidant capacity of fruits and vegetables and some pure substances [15]. As can be seen from Fig. 2, the scavenging ABTS ability of purple sweet potato is very strong whether treated or not treated with microwave, but after microwave treatment for 4 min and 8 min, the scavenging ABTS activity at the concentration of 0.02-0.04 mg / mL is almost up to 100%. When the concentration was more than 0.06 mg / mL, scavenging ABTS ability of sample treated by microwave for 8 min decreased. When the concentration was more than 0.08 mg / mL, scavenging ABTS ability would be lower than that of untreated purple sweet potato.

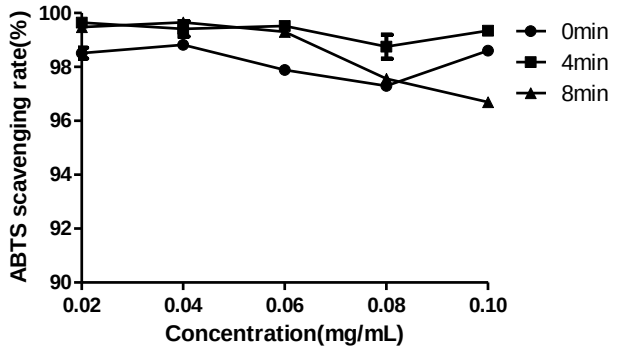


Fig.2 scanvening ABTS activity of purple sweet potato processed by different microwave

***3.3 Iron reduction ability (FRAP) determination results***

The principle of FRAP method is that the reductant in the sample can reduce Fe3 + - tripyridine triazine to Fe2 +, showing a blue color and having a maximum absorption at 593 nm. The activity of the antioxidant substance in the sample can be calculated according to the absorbance. This method is simple and easy to standardize. It is not directed against the scavenging activity of certain free radicals but rather the total reducing power of the sample. Therefore, some scholars think that the results of the FRAP method can be used to reflect the total antioxidant capacity of the sample [16]. The FRAP values of purple sweet potato after different microwave treatments are shown in the figure:

Sort according to the size of FRAP values, followed by 8 min microwave treatment> 4 min microwave treatment> untreated samples. The FRAP value of the 8 min microwave treatment sample with the strongest antioxidant capacity was 3.8 times that of the untreated sample, 1.89 times that of the 4 min microwave treatment sample, and the FRAP value of the 4min microwave treatment sample was 2.01 times that of the untreated sample.

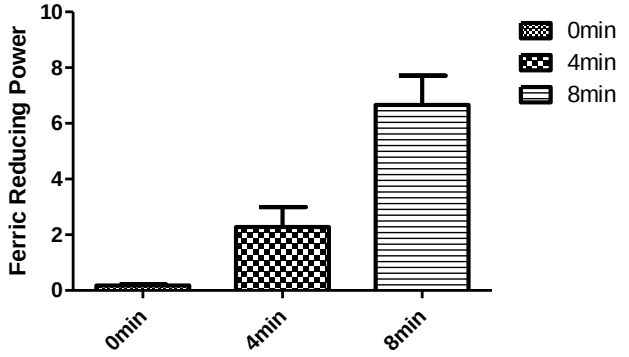


Fig.3 Total antioxidant activity (FRAP value) of purple sweet potato processed by different microwave treatment

***3.4 scavenging superoxide anion free radical (O2-) determination of the results***

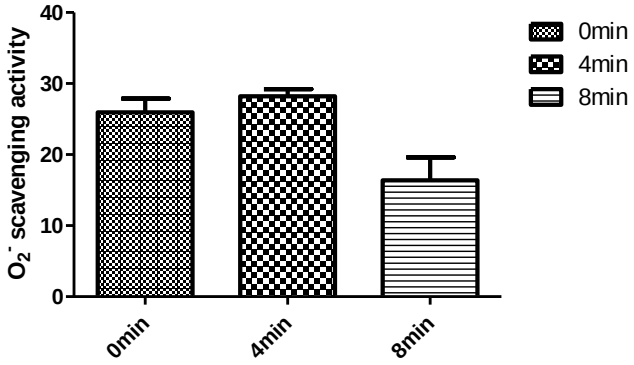


Fig.4 Scavenging superoxide anion activity of purple sweet potato processed by different microwave treatment

It is found from Fig. 4 that the ability of purple sweet potato after 4 min microwave treatment to remove superoxide anion radical is similar to that of the untreated sample, while the antioxidant capacity of purple sweet potato treated with microwave for 8 min significantly decreases, indicating that the microwave treatment for a long time has an effect on the scavenging capacity of superoxide anion.

***3.5 Determination of reducing power results***

Purple sweet potato with reducing ingredients, can reduce potassium ferricyanide to potassium ferrocyanide. Potassium ferrocyanide with $Fe^{3+}$ formed Prussian blue, which reflected the reduced strength of purple sweet potato by different treatments according to its absorbance at 700 nm. The higher the absorbance value was, the stronger the reducing ability was.

As can be seen from Fig. 5, with the increase of microwave treatment time, the reducing ability of purple sweet potato is enhanced, and the purple sweet potato reducing ability after microwave treatment is obviously enhanced compared with the untreated sample.

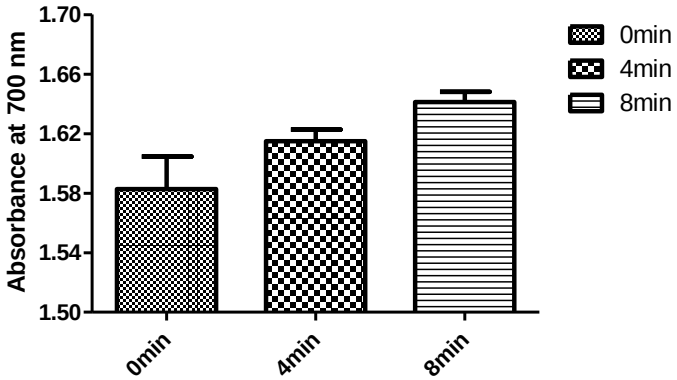


Fig.5 Reducing power of purple sweet potato processed by different microwave treatment

## 4. CONCLUSION

After purple sweet potato was processed by microwave, the antioxidant capacity of anthocyanin would be significantly improved. DPPH radical scavenging and ABTS free radical scavenging capacity and reducing power of anthocyanin in treated samples were significantly higher than untreated samples, except scavenging superoxide anion free radical of purple sweet potato treated by microwave for 8 min decreased. In short, the appropriate time microwave treatment can improve antioxidant capacity of anthocyanins in purple sweet potato.

## 5. ACKNOWLEDGEMENTS

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