The evaluation of antioxidant properties and stability of polyphenols from *Spinacia oleracea*

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Plant polyphenols have drawn increasing scientific attention due to their multiple functions on health care. In this paper, the antioxidant properties and stability of the polyphenol crude extract from *Spinacia oleracea* were studied. Two common methods including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP) assay were employed for the evaluation of antioxidant activity of spinach polyphenol. In addition, the effects of pH value, temperature, light and preservative on the antioxidant stability of spinach polyphenol were also discussed. Results showed that spinach polyphenol had a certain antioxidant activity, and its antioxidant ability increased with the increase of sample concentration. External factors, such as pH value, temperature, light and preservative, have different effects on their antioxidant stability. This study has a certain reference value for better research and utilization of plant polyphenols.

Keywords: *Spinacia oleracea*; Antioxidant activities; Polyphenols.

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Introduction

Spinacea oleracea is an important dietary vegetable popularly called spinach in China and consumed in large quantities. In 2011, the world total production of spinach was 20.79 million tons, with China alone accounting for 90.3% of this quantity [1]. Spinach is a common vegetable in the people's daily life mainly for its characteristic green color, nutritional content such as carotenes, vitamin C, and minerals such as calcium and iron [2]. The intake of a certain amount of vegetables can help reduce the risk of many diseases such as cancer and cardiovascular disease [3]. Cultivated globally, spinach is also an important raw-material in the food processing industry [4].

Recently, there is an increasing interest in the study and utilization of bioactive substances from natural sources [5]. As an important bioactive substance, plant polyphenols contain many active hydroxyl groups, which endow with a variety of physiological and pharmacological activities, such as antioxidant, antibacterial, anti-aging and so on. Therefore, the polyphenols are called as "Seventh Nutrients" [6]. Earlier studies suggested that spinach is abundant in plant polyphenols that exhibit strong antioxidant activity [7].

Antioxidants are widely used as food additives to help guard against food deterioration in the food industry. Most antioxidants are chemical compounds which are potentially dangerous for human health [8]. Hence there is a growing interest in the use of alternative plant-based natural antioxidants [9]. As a potential source of natural antioxidants, spinach polyphenol has good future prospects in food industry.

Many investigations have been reported for extraction of bioactive substance from spinach [10, 11]. However, the antioxidant properties, especially for the stability of the spinach polyphenols rarely reported. The objectives of this study were to investigate the antioxidant properties and stability of the spinach polyphenols. This study may be helpful to the further research and application of polyphenols from *Spinacea oleracea*.

Material and methods

Sample collection:

The spinach used in this study was purchased from local markets. The crude extract of spinach polyphenol was obtained by ultrasonic-assisted extraction process [12]. The extraction was performed in an ultrasonic cleaning machine Sk5200LH (frequency 40 kHz, power 320 W, manufactured by Shanghai Kedao Ultrasonic Instruments, Shanghai, China) at the temperature of 55˚C for 40 min. The dry powder of spinach (2 g) was placed into a volumetric flask (100 ml), made up to volume with ethanol: water mixtures. The ethanol concentration was 60% (v/v). After the determined time of the extraction, reaction mass was filtered, and the filtrate was collected in volumetric flask and used for the latter experiments.

Radical scavenging activity assay:

Free radical scavenging activity in the spinach polyphenol crude extract was determined using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) [13]. A 0.5 ml aqueous solution of sample extract was mixed at different concentrations with 1.0 ml methanolic DPPH (0.4 mmol/l) (Sigma Aldrich, St Louis, MO, USA). The mixture was shaken vigorously and left to stay in the dark for 60 min. The absorbance of DPPH reduction was then measured at 517 nm using UV-Vis spectrophotometer (752N, Shanghai Precision Science Instruments, Shanghai, China). All experiments were repeated three times. The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{Scavenging effect (\%)} = [1 - A_{sample}/A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control at 517 nm and A_{sample} is the absorbance of the sample at 517 nm [14].

Ferric reducing antioxidant power assay:

The total antioxidant capacity of spinach polyphenol is also commonly evaluated by another method called "Ferric Reducing Antioxidant Power" (FRAP) assay. This method is based on the ability of the antioxidants to reduce Fe^{3+} to Fe^{2+}. FRAP assay measures the change in absorbance at 593 nm due to the formation of a blue colored Fe^{2+}-tripyridyltriazine compound from colorless oxidized Fe^{3+} by the action of electron donating antioxidants [15]. FRAP reagent (Tianjin Yongchen Fine Chemical Reagent Factory, Tianjin, China) was prepared by mixing 300 mmol/l acetate buffer, pH 3.6, with 10 mmol/l TPTZ (2, 4, 6-tripyridyl-s-triazine) (Sigma Aldrich, St Louis, MO, USA) in 40 mmol/l hydrochloride acid, and with 20 mmol/l ferric chloride (volume ratio, 10:1:1). Aqueous solutions of known Fe^{2+} concentration (the $FeSO_4$ concentrations were 3.0, 2.5, 2.0, 1.5, 1.0, and 0.5 mmol/l, respectively) were used for calibration. In a reaction tube, 0.2 ml of the spinach polyphenol crude extract solution and 1.8 ml of FRAP reagent were shaken on a thermoshaker at 37°C for 10 min. Subsequently, the absorbances of the solution of samples, standards, and blank were measured at 595 nm [16]. All the experiments were performed in triplicate.

The stability of antioxidant activity:

The effects of pH, temperature, illumination, and preservative on the antioxidant activity stability of spinach polyphenol were evaluated. The antioxidant activity of spinach polyphenol was assessed by DPPH radical-scavenging activity assay. In the analysis of acid-base stability, the spinach polyphenol solution was treated for 120 min under different pH conditions. The pH values were set to 2, 4, 6, 8, and 10, respectively [17]. Determination of the thermal stability was carried out by exposing the spinach polyphenol

solution under different temperature conditions for 20-100 min [18]. The effect of light was obtained by controlling the illumination time of the sample in the incubator in the range of 1-5 hour [19]. As for the effect of preservative, the spinach polyphenol solution was treated with potassium sorbate solutions at the final concentrations of 0%, 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, respectively, for 0, 2, 4 ,6, 8 hours [20]. Each experiment was repeated three times. The data were analyzed using Analysis of Variance of statistical software SPSS (version 19.0).

Results and discussion

DPPH radical scavenging activity:

The DPPH radical scavenging activity assay provides an easy and rapid way to evaluate the antioxidant properties of antioxidants. The DPPH radical scavenging activity of spinach polyphenol was shown in figure 1. As shown, the radical scavenging effect increased with the increase of sample concentration. When the sample concentration was at 2 mg/ml, the DPPH radical scavenging effect was only 27.15%. However, the DPPH radical scavenging effect reached 76.01% when the sample concentration was increased to 10 mg/ml.

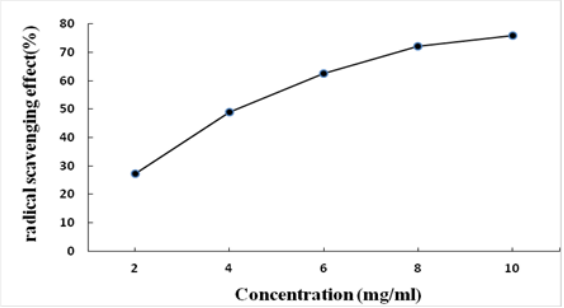

Figure 1. Radical scavenging effect of various sample concentration.

FRAP value of spinach polyphenol:

A standard calibration curve of $FeSO_4$ solution was used to calculate the FRAP value with millimoles of Fe^{2+} equivalent to per gram of spinach polyphenol. The standard curve of the $FeSO_4$ solution for FRAP assay was shown in figure 2. The standard linear equation for $FeSO_4$ solution was as follows:

$$Y = 0.4559X - 0.0985$$
$$R^2 = 0.9994$$

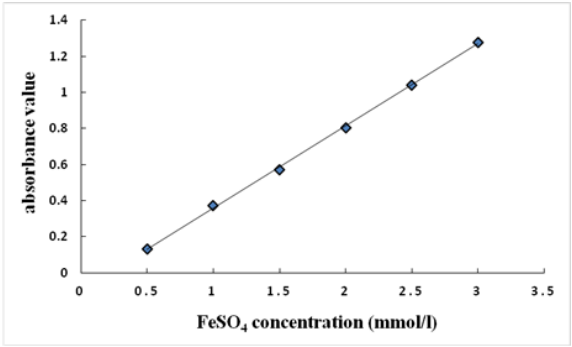

Figure 2. The standard curve of $FeSO_4$ solution for FRAP assay.

The FRAP value of spinach polyphenol was shown in figure 3. The FRAP value increased gradually with the increase of sample concentration, which indicated that the reduction activity of Fe^{2+} increased gradually with the increase of sample concentration. When the sample concentration was at 10 mg/ml, the FRAP value of spinach polyphenol reached 0.88 mmol/l. The reduction activity of Fe^{2+} depends mainly on the hydrogen atom-donating of the sample. The more the hydrogen donors are, the stronger the antioxidant activity is.

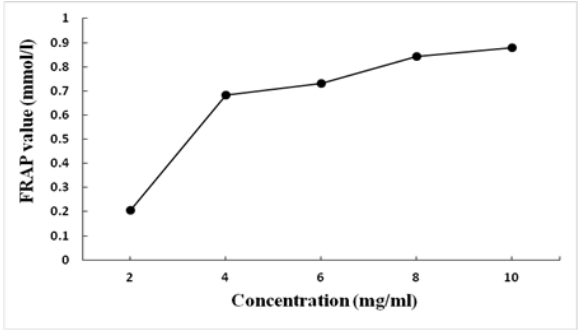

Figure 3. FARP value of spinach polyphenol.

The stability of antioxidant activity:

The effect of pH value on antioxidant activity stability of spinach polyphenol was shown in

figure 4. The antioxidant activity of spinach polyphenol changed little at the acidic condition, with the highest activity at pH 4. The antioxidant properties decreased significantly when the pH value increased, especially to the alkaline range, with the lowest activity at pH 10. This should be related to phenolic hydroxyl structure of spinach polyphenol. The increase of pH led to the partial dissociation of phenolic hydroxyl groups and the destruction of the structure. Therefore, under weak acidic conditions, the spinach polyphenol had better antioxidant activity than it under alkaline condition.

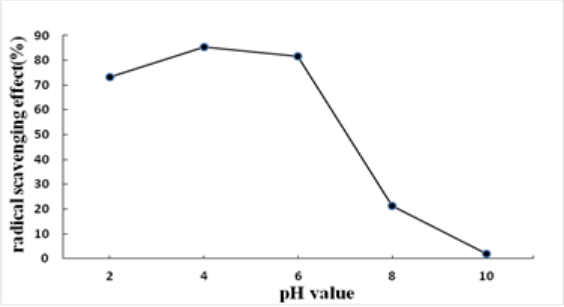

Figure 4. The effect of pH value on antioxidant activity stability.

The thermal stability of spinach polyphenol antioxidant activity was evaluated at the temperatures of 4˚C, 37˚C, 60˚C, 80˚C, and 100˚C, respectively, in conical flask with the temperature controlled by using a thermostat bath. As shown in figure 5, preserved at the temperature of 4˚C or 37˚C for a short time, the antioxidant activity of spinach polyphenol was promoted to some extent. Under other temperature conditions, the antioxidant activity decreased with the extension of the action time. Especially at 100˚C, there was a clear downward trend. This should be related to the decomposition of active substances and the destruction of the structure at high temperature.

The effect of light on the antioxidant stability of spinach polyphenol was shown in figure 6. In the continuous light group, the antioxidant activity of spinach polyphenol tended to be stable within five hours treatment. Except in dark condition for four hours, the antioxidant activity of spinach polyphenol was slightly lower. There was no significant difference among the other test sites. Therefore, we can conclude that light had little influence on the antioxidant activity of spinach polyphenol.

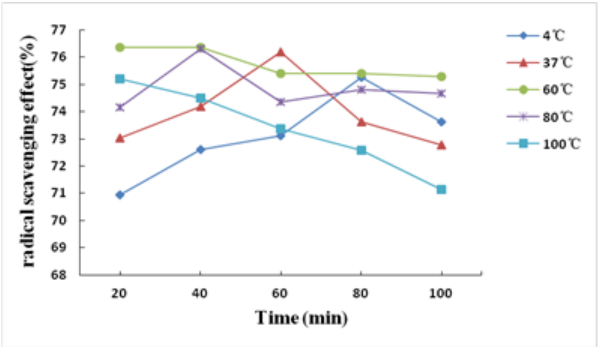

Figure 5. The effect of temperature on antioxidant activity stability.

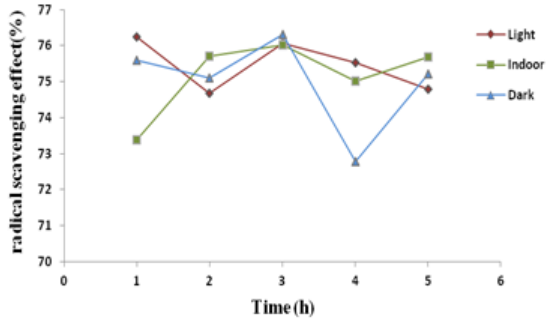

Figure 6. The effect of light on antioxidant activity stability.

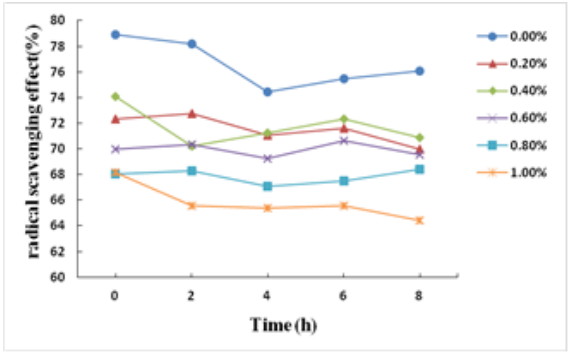

Figure 7. The effect of preservative on antioxidant activity stability.

Figure 7 showed the effect of preservative on the antioxidant stability of spinach polyphenol. In the same treatment time, the higher the concentration of preservatives was, the lower the antioxidant activity of spinach polyphenol

was. However, there was no obvious change in antioxidant activity with the extension of the action time at the same concentration. In other words, the concentration of preservatives had a significant effect on the antioxidant activity of spinach polyphenol, while the preservative action time was not obvious.

Conclusions

In recent years, the extraction and utilization of bioactive substances from the natural material have been a hotspot in both domestic and international fields [21]. Plant polyphenol is an ideal natural compound, which has many health functions such as preventing and curing disease, etc., and has potential broad application prospects [22]. The spinach is widely distributed in China. In this study, the antioxidant properties and stability of spinach polyphenol were investigated. The results showed that the spinach polyphenol had a certain antioxidant activity, and its antioxidant ability increased with the increase of sample concentration. The pH value had a significant influence on the antioxidant activity of spinach polyphenol. The spinach polyphenol had better antioxidant activity under weak acidic conditions. The antioxidant effect was especially significant at pH 4. With the increase of pH value, the antioxidant activity was decrease rapidly, and it was relatively weak under the alkaline condition. The effect of temperature on antioxidant activity was rather complicated. When the temperature was high, with the extension of the action time, there was a significant decline of antioxidant activity. Meanwhile, the results also suggested that light had little influence on the antioxidant activity of spinach polyphenol. High preservative concentration blunted the antioxidant activity of spinach polyphenol. The data obtained in this study may be useful for the further research and application of spinach polyphenol.

Acknowledgments

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