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Study on the Extraction and Antioxidant Properties of Flavonoids from Onion

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Abstract. The optimum extraction conditions and antioxidant activity of flavonoids from onion were studied. The effects of ethanol concentration, liquid-material ratio, ultrasonic power, ultrasonic time and extraction temperature on extraction rate were analyzed by single factor experiment in the extraction process. Based on the above, the optimum process conditions of onion flavonoids extraction were obtained by orthogonal experiment, namely the ethanol concentration 60%, liquid ratio 1:25, ultrasonic power 90 W, ultrasonic time 30 min, extraction temperature 40 °C, including 5.27 mg/g of flavone yield under the condition of the onion medium. *In vitro* antioxidant experiments showed that onion flavonoids had a strong reduction force, intense scavenging ability for hydroxyl radical and superoxide anion radical, and more violent antioxidant activity *in vitro* than BHT at the same concentration.

INTRODUCTION

Onion, widely planted in our country, is the seasoning vegetables ate by many east and west people, which are rich in flavonoids [1]. The production of onion has a tendency to increase per year. But some reasons, for examples, so far the consumption patterns of onion are mainly traditional cooking and small amounts of direct edible, its volume is relatively small, while the deep processing of onion also only stay in the primary level of processing, such as dehydration. Therefore, excavating fully the biofunctional factors contained in onion can promote the deep processing of onion and increase the added value of onion.

Flavonoids were a kind of common natural compounds which are widely found in natural plants, which had remarkable antioxidant and bactericidal effects [2]. In daily life, antioxidants have a very close relationship with human health. Almost all aerobic organisms produce free radicals in their normal physiological processes. But the antioxidant system in the human body will produce too much free radicals when appearing obstacles, leading to the metabolic imbalance of normal free radicals, and the occurrence of a variety of lesions. Nowadays, the common synthetic antioxidants occupied the most market. Long-term animal experiments had proved that excessive use of oxidants could cause irreversible damage to the body.

Therefore, people began to pursue green and healthy natural antioxidants [3], such as flavonoids, alkaloids and unsaturated hydrocarbons. The flavonoids studied in this paper are the signaling molecules of multi-age plants with natural antioxidants, such as biological antioxidation, scavenging free radical, anti-aging, treating cardiovascular disease, lowering blood lipid, blood pressure and blood sugar and even anticancer and certain function in medical

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health care. In addition, flavonoids can also be used as natural additives in the production of food, medicine and cosmetics [4].

At present, the main extraction methods of onion flavonoids included water extraction, ethanol extraction, microwave extraction, ultrasonic extraction and enzyme extraction. With the development of science and technology in recent years, ultrasonic assisted-extracted technology has been widely used in many industries [5]. Compared with the conventional extraction technology, the ultrasonic assisted-extracted technology is simpler in experimental equipment, and more convenient to the operation. In the process of extraction, the strong vibration, larger acceleration, strong cavitation effect and agitation can be used to accelerate the separation of the active components from the plant, which increase the yield of the active components and shorten the extraction time, avoid the destruction of high temperature to the effective components as for the traditional extraction method. At the same time, no external heating is necessary in the extraction process, which greatly reduces the loss of energy consumption.

In this paper, the flavonoids from onion were extracted by ultrasonic assisted technique. The optimum conditions were obtained by single factor analysis and orthogonal experiment. The antioxidant activity of onion *in vitro* was evaluated through comparing the reducing power of flavonoids and BHT, and eliminating ability to superoxide anion radical ($O_2^{-}\cdot$) and hydroxyl radical ($\cdot OH$), the purpose of which was to provide theoretical and experimental basis for the development and utilization of onion as a natural antioxidant and functional food.

MATERIALS AND METHODS

Materials

Red onion was bought in Jinma market in Shihezi City.

Extracted Process and Operational Key Points of Flavonoids in Onion

Fresh onion → peeling and purging → splitting → drying → crushing and screening → ultrasonic extraction → centrifuging → flavonoids extract [7]

(1) Cutting: after cleaning the surface with clean water, fresh onion peeled was cut into thin and uniform strips.

(2) Drying: Temperature is the most critical factor to drying, too high or too low temperature would loss flavonoid compounds in onions. Oven drying under 60 °C was selected in the experiments.

(3) Grinding and sifting: the onion powder from dried onion, crushed by pulverizing machine, sifted over 40 meshes is stored and lay aside.

(4) Ultrasonic extraction: a certain quality of onion powder was extracted by corresponding concentration and proportion of ethanol solution under the condition of ultrasonic wave.

(5) Centrifugation: the above extract was centrifuged for 10 min at the speed of 5 000 r/min to obtain the extract of flavonoids.

Qualitative Analysis of Flavonoids

Flavonoids were considered by color reaction [8]. Phenolic hydroxyl groups and benzopyranone ring found in flavonoids determined the properties of the color reaction. The operational process is as follows:

(1) The color reaction under visible light and ultraviolet light: after the crude extract of onion dissolved by ethanol solution was dripped on the filter paper, the color was observed under visible and ultraviolet light.

(2) HCl-Mg color reaction: this reaction is one of the most common methods detecting the flavonoids in plants. 20 mg of onion crude extract was dissolved by 1 mL of anhydrous ethanol solution, shocked further after a little Mg powder was added with dropping into a few drops of thick HCl. The color change must be observed in 1 - 2 min.

(3) $AlCl_3$ color reaction: the color reaction on filter paper was observed by adding 1% $AlCl_3$ - methanol solution into ethanol solution of onion crude extract.

Determination of the Flavonoids Yield in Onion

Preparation of Reagent

0.2 mg/mL Rutin standard solution: 10 mg of Rutin standard weighed accurately dried to constant weight, was set volume to 50 mL by deionized water, and shake well. Then, 5% Na_2NO_2:2.5 g Na_2NO_2 weighed was constant volume to 50 mL by the deionized water. Following, 10% $Al(NO_3)_3$:5 g $Al(NO_3)_3$ weighed was constant volume to 50 mL by the deionized water. Last, 4% NaOH:4 g NaOH weighed was constant volume to 100 mL by the deionized water.

Standard Curve

0.2 mg/mL of Rutin standard solution taken, 0 mL, 1 mL, 2 mL, 3 mL, 4 mL, 5 mL and 6 mL, were placed in 7 volumetric flasks (25 mL), added 1 mL 5% Na_2NO_2, respectively. The solution was mixed and allowed to stand for 6 min. 1 mL 10% $Al(NO_3)_3$ added was mixed well and for another 6 min. Finally, 10 mL 4% NaOH was added again and determined the scale by deionized water before mixing well, for 3 minutes' standing finally [9].

Absorbance of the blank solution was measured at λ=510 nm to draw the standard curve.

Determination of Flavonoid Yield of Samples

After 5 mL of onion flavonoids was accurately absorbed into a 25 mL volumetric flask, 1 mL 5% Na_2NO_2 added was mixed and stewing for 6 min. 1 mL 10% $Al(NO_3)_3$ added was mixed well for 6 min. Finally, 10 mL 4% NaOH added was mixed well again. Finally, sample was set volume to scale by deionized water for silence of 15 min [10].

Absorbance is measured at λ=510 nm with the blank reagent as the reference.The concentration was calculated according to the regression equation of standard curve, and the flavonoids yield in the sample was calculated according to the dilution factor. The calculation formula is as follows:

$$\text{Flavonoid yield}\,(\text{mg/g}) = \frac{c \times v \times n}{m} \times 4$$

Where c: flavonoid solution concentration (mg/mL); v: constant volume (mL); n: dilution ratio of onion flavonoid test solution; m: onion powder mass (g).

Determination of antioxidant activity of onion flavonoids *in vitro*

Determination of Reduction Force

Reagent preparation: PH6.6 0.2 mol/L phosphate buffer (PBS), 10% TCA solution, 1% $K_3[Fe(CN)_6]$ solution, 0.1% $FeCl_3$ solution, onion flavonoid solution and 2, 6-ditert-butyl-4-methylphenol (BHT) solution were prepared, respectively.

Assay method: Potassium ferricyanide ($K_3[Fe(CN)_6]$) reduction method was used under control BHT. Flavonoid solutions and BHT solutions were prepared according to gradients (0.1 mg/mL, 0.2mg/mL, 0.3mg/mL, 0.4 mg/mL, 0.5mg/mL and 0.6 mg/mL).

Specific operations are shown in Table 1.

TABLE 1. Operation steps of $K_3[Fe(CN)_6]$ reduction method

	Blank tube	Tube
Distilled water (mL)	5	0
Flavone or BHT (mL)	0	5
PBS, 1% $K_3[Fe(CN)_6]$ (mL)	2.5 each	2.5 each
20 min water bath under 50 °C before cooling rapidly		
10% TCA (mL)	2.5	2.5
3000 rpm for centrifugation under 10 min before 4 mL supernatant was took		
Distilled water (mL)	5	5
0.l% $FeCl_3$ (mL)	1	1
The light absorption value was measured at 710 nm after being mixed for 10 min		

Determination Scavenging Capacity of Hydroxyl Radical (·OH)

Preparation of Reagents

Different concentration gradients of flavonoid solutions and BHT solutions (0.04 mg/mL, 0.08 mg/mL, 0.12 mg/mL, 0.16 mg/mL and 0.20 mg/mL), 6 mmol/L ferrous sulfate solutions ($FeSO_4$), 6 mmol/L H_2O_2, 6 mmol/L salicylic acid [11-12] were prepared, respectively.

Determination Method

Salicylic acid method was employed. Specific operations were shown in Table 2.

TABLE 2. Operation steps of salicylic acid method

	Blank tube (A_0)	**Sample background tube (A_1)**	**Sample cell (A_2)**
Flavone or BHT (mL)	0	2	2
$FeSO_4$ (mL)	2	2	2
H_2O_2 (mL)	2	2	2
Mix well and stewing for 10min			
Salicylic acid (mL)	2	0	2
Distilled water (mL)	2	2	0
The light absorption value was measured at 510 nm after being mixed for 10 min			

Formula for calculating clearance rate:

$$\text{Clearance rate of } \bullet\text{OH}\,(\%) = \left(1 - \frac{A_2 - A_1}{A_0}\right) \times 100\%$$

Where A_0: absorbance of control tube without samples; A_1: absorbance of sample background tube without reagent and sample; A_2: absorbance of sample tubes with reagent and sample.

Determination of the Scavenging Capacity of Superoxide Free Radicals ($O_2^{-}\cdot$)

Reagent Preparation

Different concentration gradients of flavonoid solutions and BHT solutions (0.04 mg/mL, 0.08 mg/mL, 0.12 mg/mL, 0.16 mg/mL and 0.20 mg/mL), 45 mmol/L catechol, ph8.2 50 mmol/L Tris-HCL buffer, 3 mol/L hydrochloric acid were prepared [13], respectively.

Determination Method

Phloroglucinol method was used for detection. Specific operations were shown in Table 3.

TABLE 3. Operation steps of catechol method

	Blank tube (A_0)	**Sample background tube (A_1)**	**Sample cell (A_2)**
Tris-HCl (mL)	5	5	5
Flavone or BHT (mL)	0	5	5
Pyrogallol (mL)	1	0	1
Distilled water (mL)	5	1	0
The light absorption value was measured at 300 nm after being mixed with 3 moL/L HCl 1mL for 5 min			

Formula for calculating inhibition rate

$$\text{Inhibition rate of } O_2^{-}\bullet\,(\%) = \left(1 - \frac{A_2 - A_1}{A_0}\right) \times 100\%$$

Where A_0: absorbance of control tube without samples; A_1: absorbance of sample background tube without reagent and sample; A_2: absorbance of sample tubes with reagent and sample.

RESULTS AND DISSCUSS

Rutin Standard Curve

The Rutin standard curve was drawn by plotting the concentration of Rutin standard solution (abscissa) versus the corresponding absorbency (ordinate) by linear regression of least square method, as shown in Fig. 1. The regression equation was y=11.98x-0.008, R^2=0.999, where y was the absorbance of the sample, x was the concentration of Rutin, and R^2 of this equation was 0.999.

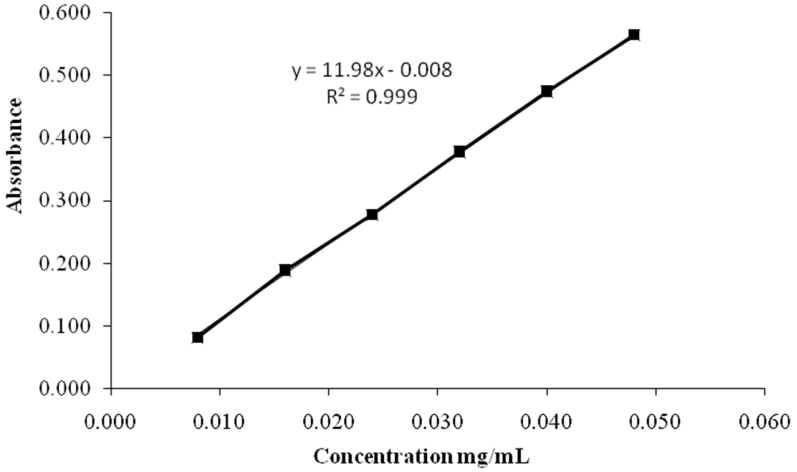

FIGURE 1. Standard curve of Rutin

Qualitative Experiment of Flavonoids

The results of chromogenic reaction were shown in Table 4.

TABLE 4. Results of chromogenic reaction

	Visible light	Ultraviolet light
Extract	Claybank	Yellow, fluorescent
Chromogenic reaction of HCL-Mg	Yellow	Yellow and green, fluorescent
$AlCl_3$ chromogenic reaction	Yellow	Yellow, fluorescence

Chromogenic reaction confirmed that flavonoids were contained in the extract, but the specific components needed be measured by separation and purification further.

Single Factor Experiment for Flavonoids Extraction from Onion

In this experiment, the effects of ethanol concentration, liquid-material ratio, ultrasonic power, ultrasonic time and extraction temperature on the yield of flavonoids in Onions were investigated, and the yield of flavonoids in Onions was taken as the measured index [14].

Influence of Ethanol Concentration on Flavonoid Yield

Extraction of organic solvent is the widest method for flavonoids extraction at home and abroad. The commonly used organic solvents include methanol, ethanol, acetone, butanol, petroleum ether, ethyl acetate, etc. Flavonoids are highly soluble in methanol and ethanol solvents, but ethanol was used as the extraction solvent in this experiment owe to highly toxic methanol [15].

1.0 g dried onion powder was extracted with feed liquid rate 1:20 and ultrasonic power 70 W under six levels of ethanol concentration, 30%, 40%, 50%, 60%, 70% and 80%, for 30 min at a controlled 35 °C. The sample was

extracted for 3 times in total. Effective results of different ethanol concentration on the yield of onion flavonoids were shown in Fig. 2.

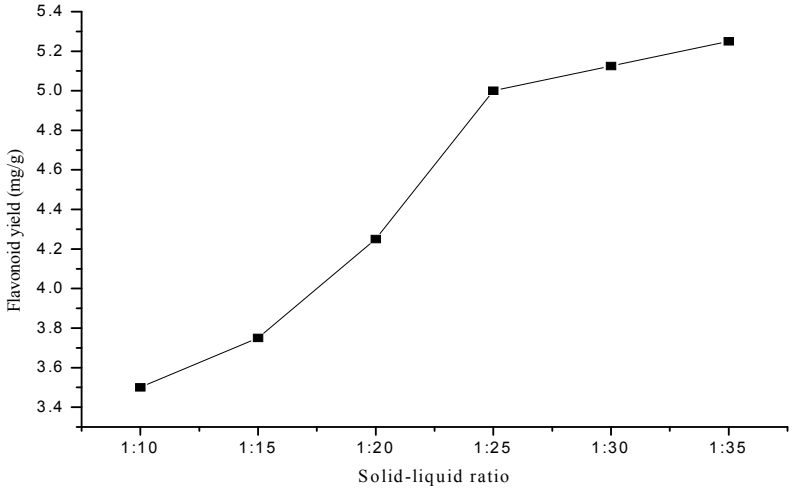

FIGURE 2. Effect of ethanol concentration on flavonoid yield

From Fig. 2, the yield increased rapidly with the rise of the ethanol concentration under the lower level of ethanol. The yield of flavonoids in onion reached the maximum, 5.25 mg/g, at the 60% concentration. Then, the yield of flavonoids decreased along with further increase of the ethanol concentration. The flavonoid yield was only 3.375 mg/g once the concentration of ethanol increased to 80%. The reason why the flavonoid yield in onion increased before decreased with the augment of ethanol concentration was obvious that ethanol has good solubility and strong cell penetration. The higher the concentration of ethanol was, the greater the differential concentration between ethanol and onion tissue, the better the yield. However, the excessive concentration of ethanol will increase the dissolution of some alcohol-soluble impurities, pigments and lipophilic components, which will compete with ethanol-water molecules and cause interference to the experiment, thus leading to the decrease of flavonoid yield in onion. Therefore, 60% ethanol concentration was appropriate.

Effect of Solid-Liquid Ratio on Flavonoid Yield

1.0 g dried onion powder was extracted with 60% ethanol and 70 W ultrasonic power under six levels of feed liquid rate, 1:10, 1:15, 1:20, 1:25, 1:30, and 1:35, for 30 min at a controlled 35 °C. The sample was extracted for 3 times in total. Effective results of different feed liquid rate on the yield of onion flavonoids were shown in Fig. 3.

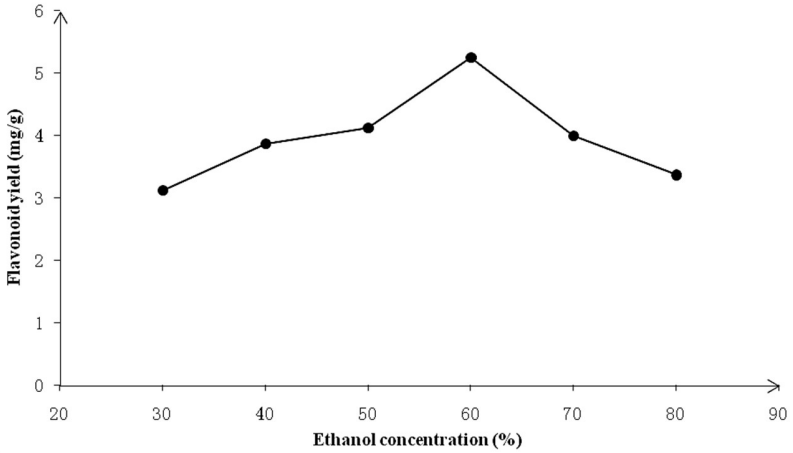

FIGURE 3. Effect of solid-liquid ratio on flavonoid yield

From Fig. 3, the flavonoid yield of onion was 3.5 mg/g when the solid-liquid ratio was 1:10. When the solid-liquid ratio reached 1:25, the flavonoid yield was upto 5 mg/g, with a significant increase. When the ratio of feedstock to liquid increased continually to 1:30 and 1:35, the flavonoid yield began to rise slowly. Generally speaking, the higher the ratio of material to liquid was, the higher the extraction rate. However, when the ratio increases to a certain extent, a certain proportion of the solvent has basically dissolved the active components out, so the yield tends to be stable. Moreover, excessive ethanol consumption will cause waste of organic solvents and make the subsequent concentration time too long. Therefore, from the perspective of extraction rate, solvent dosage and production cost, it was advisable to choose the ratio of material to liquid at 1:25.

Influence of Ultrasonic Power on Flavonoid Yield

1.0 g dried onion powder was extracted with 60% ethanol and feed liquid rate 1:20, under six levels of ultrasonic power, 50 W,60 W,70 W,80 W,90 W and 100 W, for 30 min at a controlled 35 °C. The sample was extracted for 3 times in total. Effective results of different ultrasonic power on the yield of onion flavonoids were shown in Fig. 4.

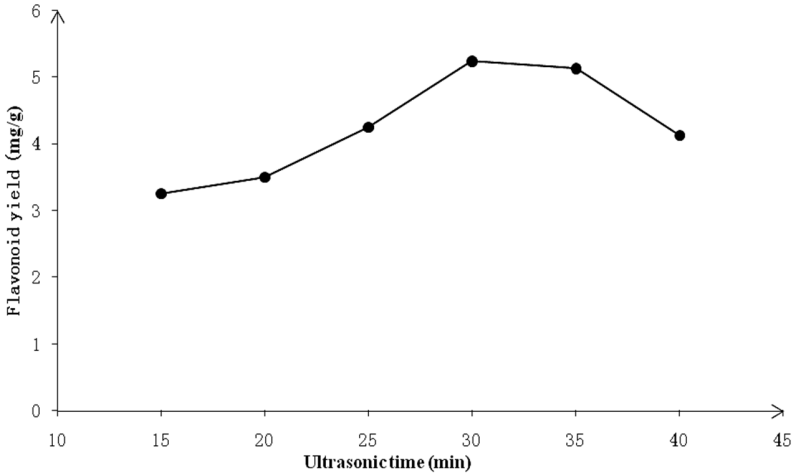

FIGURE 4. Influence of ultrasonic power on flavonoid yield

It can be seen from Fig. 4 that the flavonoid yield of onion increased with the enlargement of ultrasonic power. When the ultrasonic power was adjusted to 90 W, the flavonoid yield of onion reached the maximum. However, the flavonoid yield began to reverse when the ultrasonic power surpassed 90 W, which derived from too higher the ultrasonic power leading degradation of flavonoid and increase of dissolution level on other impurities. Therefore, it was optimal to select 90 W ultrasonic powers.

Effect of Ultrasonic Time on Flavonoid Yield

1.0 g dried onion powder was extracted with 60% ethanol and feed liquid rate 1:20, under six levels of extracting time, 15 min, 20 min, 25 min, 30 min, 35 min and 40 min, for ultrasonic power 90 W at a controlled 40 °C. The sample was extracted for 3 times in total. Effective results of different extracting time on the yield of onion flavonoids were shown in Fig. 5.

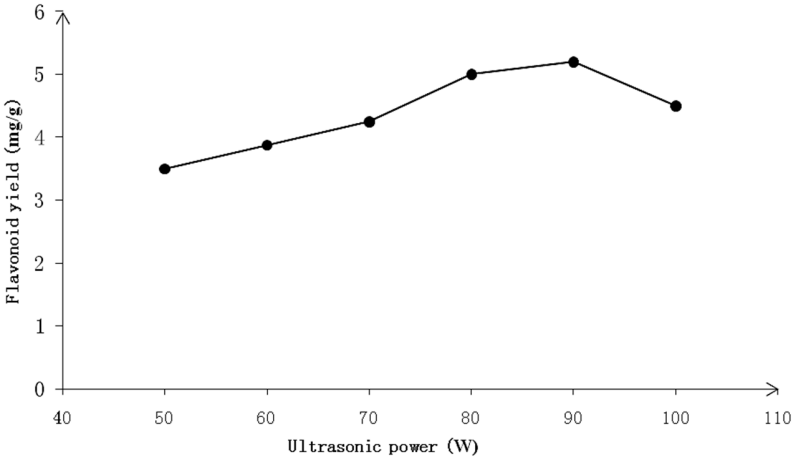

FIGURE 5. Effect of ultrasonic time on flavonoid yield

As can be seen from Fig. 5, the flavonoid yield of onion was 3.25 mg/g at the 15 min of extraction time. With the extension of the extraction time, the flavonoid yield of onion increased gradually but slowly. When the extraction time extended to 30min, the flavonoid yield reached 5.24 mg/g. However, the yield increased more slowly with the extension of extraction time further. At the beginning of extraction, the difference of concentration between the raw material and the extraction solution aggrandized the mass transfer force, which led to increase the yield rapidly. After a certain time, the flavonoids concentration inside and outside the raw material reached a state of relative equilibrium, and the flavonoids inside the raw material were not easy to be leached continually. In addition, properties of flavonoids with antioxidant but destroyed easily by oxidation should be considered at the process of extraction. Prolonging exposure to flavonoids, especially when high temperature extraction, may lead to reduce yield and antioxidant activity. Therefore, the extraction time cannot be extended blindly even though in order to improve the yield. The ultrasonic time of 30min was appropriate.

Influence of Extraction Temperature on Flavonoid Yield

1.0 g dried onion powder was extracted with 60% ethanol and feed liquid rate 1:20, under six levels of extracting temperature 30 °C, 35 °C and 40 °C, 45 °C, 50 °C and 55 °C, under ultrasonic power 90 W for ultrasound time 30 min. The sample was extracted for 3 times in total. Effective results of different extracting temperature on the yield of onion flavonoids were shown in Fig. 6.

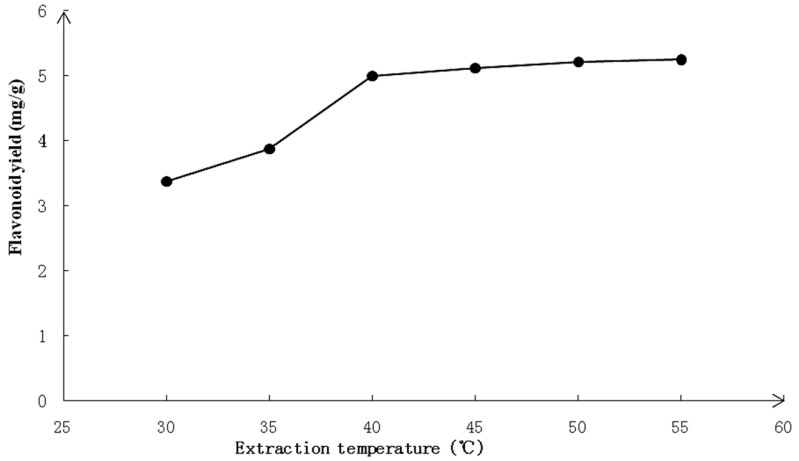

FIGURE 6. Influence of extraction temperature on flavonoid yield

From Fig. 6, the influence of temperature on the yield was the larger. Onion flavonoids yield increased obviously with extracting temperature between 30 - 40 °C. The flavonoid yield of 5 mg/g appeared the extraction temperature of 40 °C. However, extraction yield increased with the continual rise of temperature, the growth rate was reduced yet. The flavonoid yield broke through 5.25 mg/g, higher than only 0.25 mg/g at 40 °C, upto 55 °C. So 40 °C was advisable choose to extraction temperature.

Generally speaking, the higher the temperature was, the higher the average movement speed of a substance to a molecule would be, which increased the diffusion rate. In addition, the increase of temperature also promoted the infiltration of plant tissue and the dissolution of effective components, which could coagulate proteins in plants and destroy enzymes to facilitate the leaching of substances. However, too high temperature would accelerate the decomposition or isomerization of the active ingredients, so the temperature should not be too high for flavonoids with reducibility as active substances [16].

From Fig. 6, the biggest change of flavonoids yield in onion was about 30 - 40 °C, because the whole extraction process was affected by infiltration and mass transfer driving force in addition to temperature, and effect of temperature on infiltration, dissolution and diffusion existed otherness. With the ascending temperature, comprehensive promotion capacity increased, nevertheless the increase of extraction rate was quicker. With the further increase of temperature, the mass transfer process was basically determined by a certain control step, and the promotion effect of other factors was no longer obvious, so the increasing trend of extraction rate became slow.

Orthogonal Experiment of Flavonoids Extraction from Onion

According to the results of single factor experiment, four single factors influencing the experiment were selected as the investigative factors, meanwhile three levels were taken near the optimal value of each factor, and the orthogonal table $L_9(3^4)$ was used for the design. Finally, four factors including ethanol concentration, solid-liquid ratio, ultrasonic power and ultrasonic time were selected for orthogonal experiment of 4 factors and 3 horizons to determine the optimal technological conditions for flavonoids extraction from onion [10].

The factor level table and the results on orthogonal experiment were shown in Table 5 and 6.

TABLE 5. Factor level table of orthogonal experiment $L_9(3^4)$

	Factor			
Level	**A**	**B**	**C**	**D**
	Ethanol concentration (%)	**Solid-liquid ratio (g/mL)**	**Ultrasonic power (W)**	**Ultrasonic time (min)**
1	50	1:20	80	25
2	60	1:25	90	30
3	70	1:30	100	35

TABLE 6. Results of orthogonal test

The experiment number	**A**	**B**	**C**	**D**	**Flavonoid yield (mg/g)**
1	1	1	1	1	3.250
2	1	2	2	2	3.125
3	1	3	3	3	4.250
4	2	1	2	3	5.125
5	2	2	3	1	4.500
6	2	3	1	2	5.238
7	3	1	3	2	3.875
8	3	2	1	3	3.500
9	3	3	2	1	4.125
k1	3.542	4.083	3.996	3.958	
k2	4.954	3.708	4.125	4.079	
k3	3.833	4.538	4.208	4.292	
R	1.412	0.829	0.212	0.333	

According to the results of range analysis in Table 6, the optimal process conditions extracted were $A_2B_3C_3D_3$, namely 60% ethanol, 1:25 liquid-material ratio, ultrasonic power 90 W and ultrasonic time 30 min. The effects of various factors on the extraction rate of flavonoids from onion were A> B> D> C according to the primary and

secondary relationships, namely ethanol concentration > feed liquid rate > ultrasonic time > ultrasonic power. The yield of flavonoids extracted from onion was 5.27 mg/g according to the optimal extraction conditions.

Antioxidant Activity of Flavonoids *in vitro*

Reduction Capacity

There was a relationship between reducing power and antioxidant activity of antioxidant [17]. Antioxidants could scavenge free radicals through own reduction providing electrons. The stronger the reduction capacity of samples was, the better the antioxidant capacity. Therefore, the antioxidant activity could be expressed by measuring the reduction force of samples.

Potassium ferricyanide ($K_3[Fe(CN)_6]$) reduction method was employed in this experiment [11], the principle of which was that after antioxidants reduced ferric iron (Fe^{3+}) to ferric bivalent (Fe^{2+}), Fe^{2+} formed soluble blue complex $KFe[Fe(CN)_6]$ with $K_3[Fe(CN)_6]$ with the maximum absorption at 710 nm. From Fig. 7, onion flavonoids in low concentration could generate a high reduction force, which increased gradually with the increase of concentration. The absorbance value of onion flavonoids was 2.24 under the 0.1 mg/mL, and 2.75 under 0.6 mg/mL. It was also known that the reduction force of onion flavonoids was much higher than that of the control BHT. The absorbance value of BHT was only 0.21 under 0.1 mg/mL, but 0.69 under 0.6 mg/mL, which indicated that the antioxidant capacity of onion flavonoids was better than that of BHT.

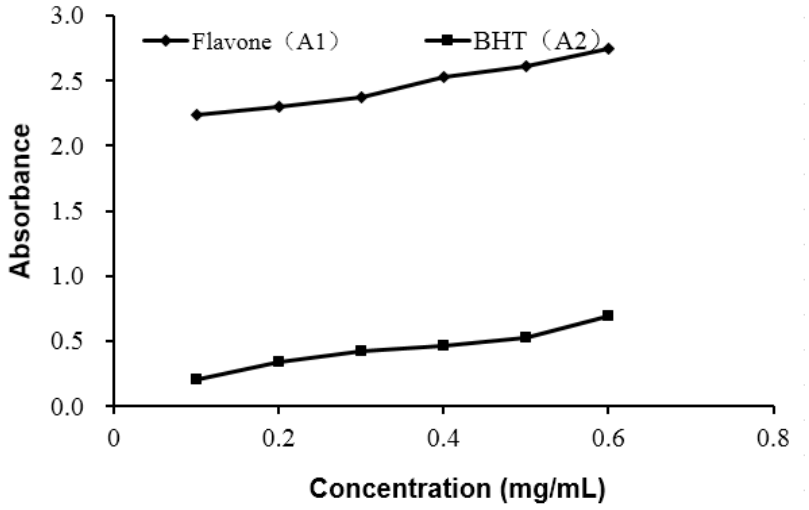

FIGURE 7. Determination of flavonoid reducing power of onion

Ability to Scavenge Hydroxyl Radical (·OH)

Hydroxyl radical is a free radical eliminated difficultly with the most toxic and harmful living organisms in reactive oxygen species, which could destroy many active components such as polysaccharides, lipids and amino acids in cells, cause cell necrosis or mutation [12]. Therefore, the scavenging effect of hydroxyl radical was very important on antioxidant activity of samples in the study.

This experiment adopted the method of salicylic acid [13]. The basic principle of method was that hydroxyl free radicals produced hydroxyl compounds through attacking vulnerably aromatic compounds. Therefore, ·OH in the Fenton reaction system was captured by salicylic acid. The 2, 3-dihydroxybenzoic acid generated was extracted with ethyl ether, and the color was developed by sodium tungstate and sodium nitrite. Then, the absorption value at 510nm was determined by spectrophotometer, which could reflect the concentration of hydroxyl radical in the system.

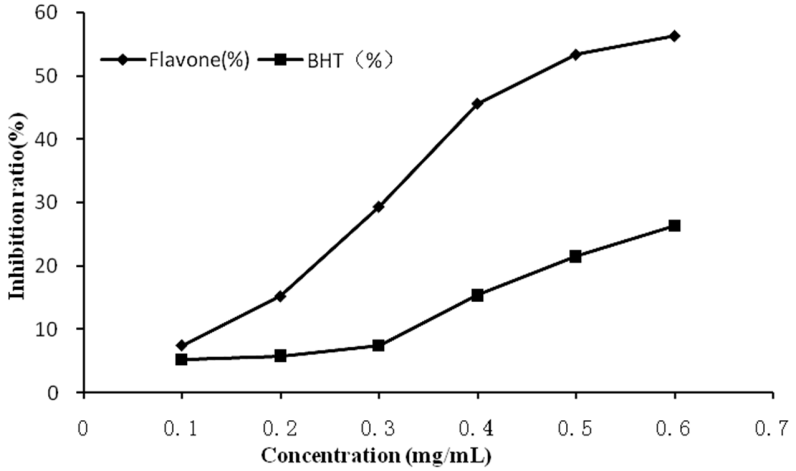

FIGURE 8. Determination of ability of scavenging hydroxyl radicals (·OH) on flavonoid in onion

From Fig. 8, the scavenging effect of onion flavonoids on ·OH could be observed at all concentrations, and the scavenging effect increased gradually with the increasing concentration. In addition, the scavenging ability of flavonoids from onion was higher than that of control substance BHT. The clearance rate was only 7.46% under 0.1 mg/mL of flavonoid concentration, and increased to 56.27% under 0.6 mg/mL the concentration. However, the clearance rate was only 5.33% as for the 0.1 mg/mL BHT, and increased to 26.40% under 0.6 mg/mL concentration. The scavenging rate of flavonoids was more than 30% compared with that of BHT at 0.6 mg/mL, indicating that onion flavonoids had a significant scavenging effect on ·OH.

Ability to Remove Superoxide Free Radicals (O_2^-)

Superoxide anion, the longest free radical in the body, was usually used as the initiator of free radical chain reaction and could produce other free radicals through a series of reactions, cause damage to the body further [18]. Therefore, the scavenging ability of samples is usually regarded as an important indicator of antioxidant activity.

Catechol method was used in this experiment [19]. The basic principle of pyrocatechol method is that pyrocatechol will be oxidized automatically and release $O_2^{-\cdot}$ under alkaline conditions, and self-oxidation process of $O_2^{-\cdot}$ can generate colored intermediate products further. With the continuous oxidation of intermediate products, the reaction liquid will turn yellow-brown before turning green in a few minutes and turning yellow in a few hours, successively. The intermediate has strong light absorption at 300 nm in the initial stage of catechol self-oxidation. $O_2^{-\cdot}$ can combine with H^+ to generate O_2 and H_2O_2 by catalytic action of antioxidants, which prevent the accumulation of intermediate products. Therefore, the stronger the ability to remove $O_2^{-\cdot}$ is, the lower the absorption value of light.

From Fig. 9, the flavonoids in onion had all scavenging effect on $O_2^{-\cdot}$, and the scavenging effect increased gradually with the increasing concentration. In addition, the ability of onion flavonoids to remove $O_2^{-\cdot}$ was higher than that of control BHT. When the flavonoid concentration was 0.1 mg/mL, the clearance rate was only upto 16.23%. While the clearance rate increased to 28.15% under the 0.6 mg/mL flavonoid concentration. As for BHT, the clearance rate was only 2.31% as for the 0.1 mg/mL concentration, and increased to 6.29% when the concentration was 0.6 mg/mL, which was less than 22% compared with that of flavonoids, indicating that onion flavonoids had a significant scavenging effect on $O_2^{-\cdot}$.

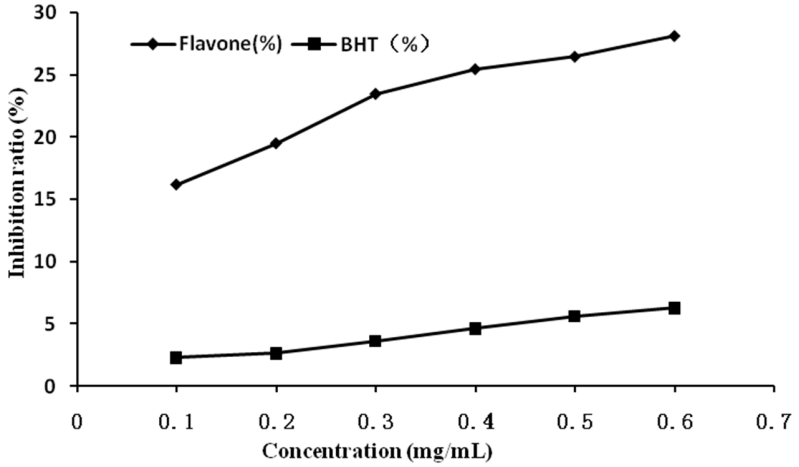

FIGURE 9. Determination of ability of scavenging O_2^- on flavonoid in onion

During the extraction of flavonoids in this experiment, it was found that the crude extractions contained non-target flavonoids, such as proteins, lipids, sugars and pigments. In order to ensure the purity of the target flavonoids, the differences between flavonoids and other substances should be clarified, and proper separation and purification of crude flavonoids should be carried out by certain technological means [20-21].

In this experiment, reducing capacity of flavonoids were only compared with that of BHT, while antioxidant activity focused merely on capacity of hydroxyl radical (·OH) and superoxide anion radical ($O_2^{-}\cdot$). Previous studies had found that the reducing capacity to Vc [22] and the removing ability to diphenyl bitter umbilical radical (DPPH·) [23] could also reflect the antioxidant activity of flavonoids *in vitro*.

Flavonoids compounds cannot be synthesized directly in human body, except from natural food. Therefore, the technological research on flavonoids extraction and separation is very important. With the development of modern separative technologies, some technologies, such as ultrasonic extraction and microwave extraction would be widely used in the extraction and separation of onion flavonoids [24]. The further research on the extraction and antioxidant activities of flavonoids from Onions would help to develop the application of flavonoids in natural food, such as antioxidants and anti-aging, cardiovascular diseases, as well as lowering blood lipid, blood pressure and blood glucose and even anticancer drugs [25].

CONCLUSIONS

The best process conditions extracting onion flavonoids by the assistive technology of ultrasonic, integrated single factor and orthogonal test results were: 60% ethanol, the ratio of liquor to material 1:25, ultrasonic power 90 W, ultrasonic time 30 min, and extraction temperature 40 °C. The flavonoids yield on onion was 5.27 mg/g. Onion flavonoids had antioxidant activities and better reduction force. Compared with BHT at the same concentration, onion flavonoids could effectively eliminate hydroxyl radical (·OH) and superoxide anion radical ($O_2^{-}\cdot$).

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