Experimental Study on the Scavenging Effect of Chestnut Flower Extract on Nitrite

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Abstract. This paper aims to reveal the scavenging effects of chestnut flower extract on nitrite. The effects of extraction temperature, extraction time, ethanol concentration on the removal effect of nitrite were studied by single factor test and orthogonal test. The best conditions for removing nitrite in the result were shown as below: extraction temperature: 40 ℃, ethanol concentration: 40%, extraction time: 1.5 h, and the maximum scavenging rate could reach 94% are the optimal conditions for its reaction with nitrite.

1. Introduction
Chesnut flower is the male inflorescence of chestnut in the genus castanea of the fagaceae, chestnut flower belongs to mild-natured, non-toxic. According to the Chinese traditional medicine dictionary records, chestnut flower has the effect of treating blood in stool, long red and white dysentery, large intestine, diarrhea and indigestion in children. Also, chestnut flower contains flavonoids, estradiol, polysaccharide and other active substances. Chestnut flowers, in particular, have the highest concentration of flavonoids of all pollens [1-2]. The male flower of chestnut has numerous benefits, such as enhancing vascular tension, reducing the formation of thrombosis; besides, it can also protect liver and detoxify. Furthermore, chestnut flower can improve immunity, anti-bacterial, anti-virus, anti-oxidation, anti-aging, anti-fungal, anti-tumor and so on[3]. Chinese chestnut belongs to monocious fruit trees, and the ratio of male and female flowers is between 1 : 2400-1 : 4000[4]. Therefore, a large number of male flowers can be produced, especially in recent years, with the continuous increase of chestnut planting area, a large number of chestnut flowers will be produced every year. The research shows that chestnut flower has the value of development and application in many fields such as food, medicine and health care products[5-6]. As a result, the research and development of chestnut flowers have important social and economic benefits.

With the development of science, technology and the improvement of people’s living standards, people start to pay more attention to healthy diet. Although Nitrite content in fresh food is low, nitrite...
content will gradually increase due to the prolongation of storage time. Research shows that nitrite can bind with secondary amines, form nitrosamines, induce canceration of digestive system, and pose a threat to human health[7-8]. Therefore, the best condition of removing nitrite from chestnut extract was explored to provide reference for people's diet.

In the last few decades, there were several research on chestnut flowers. For example, Wang S et al[9] studied the chemical constituents of chestnut flower[10]. The content of flavonoids in chestnut flower was determined by colorimetry. The content of flavonoids in chestnut flower was much higher than that in other parts of chestnut. Li Y J et al. found that the extraction process and antibacterial activity of chestnut realflavone, and chestnut flower had inhibitory effects on bacillus subtilis, escherichia coli and yeast of beer[11]. In the study of the antioxidant activity of chestnut flower crude extract, Wu X H et al. found that Chestnut flower crude extract can effectively remove DPPH· and OH radicals[12].

2. Materials and Reagent

2.1 Materials
Chestnut flower was provided by chestnut planting resource base in Huai Rou district, Beijing.

2.2 Reagents
Ethanol and hydrochloric acid were purchased from Beijing Chemical Plant, China. Ethanol is the analytical grade and hydrochloric acid is the superior grade.

3. Experimental Methods

3.1 Preparation of standard nitrite solution
0.05 g of sodium nitrite was added to 250 mL capacity. The standard solution of sodium nitrite was prepared by injecting distilled water into the bottle to scale. The above standard solution of 6.25 mL was injected into 250 mL volumetric flask, and distilled water was added to prepare the standard sodium nitrite solution with a concentration of 5 μg/mL[13].

3.2 Determination of the maximum absorption wavelength
First, 2.5mL 5 μg/mL NaNO2 standard solution was absorbed and placed in a 10 mL colorimetric tube, add 2 mL of 0.4% sulfanilic acid, shake well and place for 4 min. Then 0.2% naphthalene ethylenediamine hydrochloride was added 1 mL  and constant volume, sitting for 15 minutes. The reaction solution was poured into a 1cm cuvette, and the maximum absorption wavelength measured by spectral scanning was 544 nm[14].

3.3 Test for determination of nitrite removal rate
A certain amount of chestnut flower extract was placed in a 25 mL colorimetric tube, then add 4 mL 5 μg/mL NaNO2 and 5mL pH=3 sodium citrate buffer. After that 4 mL nitrite was added in a constant temperature water bath at 90 ℃ for 70 min to remove nitrite. The other was a blank test. After the reaction, 0.4% aminosulfonic acid 2 mL was added into the colorimetric tube and then blended and kept for 4 minutes. The next step is to add 1 mL of 0.2% naphthalene ethylenediamine hydrochloride, shake well in constant volume, and stand for 15 minutes. Absorption A01 was measured at 544 nm, and the blank was A02. Repeated three times to take the average, nitrite removal rate according to the formula.

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\text{Nitrite scavenging rate } \% = \frac{(A02 - A01)}{A02} \times 100
\]  

3.4 Single-Factor Experiment
The effects of ethanol concentration, extraction time and extraction temperature on nitrite removal
from chestnut flower extract were studied. The extractions were carried out at different concentrations of ethanol (20%, 40%, 60%, 80%, 100%) with different extraction time (0.5 h, 1 h, 1.5 h, 2 h, 2.5 h) and different extraction temperatures (20 ℃, 40 ℃, 60 ℃, 80 ℃, 100 ℃). The extracted extract was filtered and stored in sealed shelter. According to the effect of removing nitrite from the extract, the extraction effect of the extract was judged.

3.5 Orthogonal Design
Nine 1 g chestnut flowers were selected as indicators of nitrite removal rate. Temperature, ethanol concentration and extraction time were selected as factors. The effects of different extraction temperature, ethanol concentration and extraction time on nitrite removal rate were studied according to L9 (34) orthogonal table. As shown in the table 1, each factor has three levels.

| Levels | A Temperature/℃ | B Solvent Concentration/% | C Time/h |
|--------|------------------|---------------------------|---------|
| 1      | 40               | 40                        | 1.5     |
| 2      | 60               | 60                        | 2       |
| 3      | 80               | 80                        | 2.5     |

4. Results and Discussion

Figure 1. Effect of ethanol concentration on removal rate of nitrite
As shown in figure 1, the chestnut flower extract extracted with a low concentration of ethanol solution has a better effect on nitrite removal. With the increase of ethanol concentration, the removal effect increased too. When ethanol concentration reaches 60%, its removal effect is the greatest. However, with the increase of ethanol concentration, its removal effect of nitrite shows a downward trend. It may be that some impurities were extracted at high concentration to influence the absorbance value of the experimental reaction. At the same time, it can be shown that 60% ethanol can produce more active ingredients with nitrite.
Figure 2. Effect of extraction time on nitrite removal rate

As can be seen from figure 2, the effect of extraction time on nitrite removal is not significant. When the extraction time was 2 hours, the extract had the best effect on nitrite removal. With the continuous increase of extraction time, the scavenging effect of the extract began to decline. The results showed that the nitrite-scavenging active ingredients in chestnut flowers had basically been completely dissolved at 2 h of extraction time.

Figure 3. Effect of extraction temperature on nitrite scavenging rate

It could be seen from figure 3 that as the extraction temperature, the removal rate of nitrite from chestnut flower extract increases, and when the temperature reaches 60 °C, the removal rate reaches a maximum. Then, as the temperature continues to rise, the nitrite scavenging rate shows a downward trend. The results showed that the optimum extraction temperature for nitrite scavenging was 60 °C, and the scavenging rate could reach 93%. However, with the increase of temperature, some of the free radicals in the extract might be inactivated, thus reducing the ability of nitrite scavenging.

Table 2. Orthogonal test was used to optimize the extraction process for removing nitrite from chestnut flower extract.

| No. | A  | Empty column | B  | C  | Nitrite removal rate /% |
|-----|----|--------------|----|----|--------------------------|
| 1   | 1  | 1            | 1  | 1  | 93.83                    |
| 2   | 1  | 2            | 2  | 2  | 88.39                    |
| 3   | 1  | 3            | 3  | 3  | 71.39                    |
| 4   | 2  | 1            | 1  | 2  | 89.53                    |
Table 3. Analysis of Variance of Extracting Technology.

| Source                | SS     | df | F     | P     |
|-----------------------|--------|----|-------|-------|
| Temperature           | 111.577| 2  | 1.504 | >0.05 |
| Ethanol concentration | 1991.517| 2 | 26.846| <0.05 |
| Temperature           | 102.277| 2  | 1.379 | >0.05 |
| Error                 | 74.183 | 2  |       |       |

Comparing the range R of the three factors, it is obvious that the concentration of ethanol is the most important factor for removing nitrite from the extract, and the difference is significant by variance analysis (p < 0.05), followed by extraction time and extraction temperature. According to the table, the optimum combination of experimental conditions for removing nitrite from chestnut flower extract is as follows: extraction temperature: 40 °C, ethanol concentration: 40%, extraction time: 1.5 h. The nitrite removal rate after verification by three parallel experiments was 94%.

5. Conclusion
The extract of chestnut flower had a good scavenging effect on nitrite. The optimum conditions for the removal of nitrite by the extract were as follows: extraction temperature: 40 °C, ethanol concentration: 40%, extraction time: 1.5 h, at the moment the removal rate could reach 94%. According to reports, flavonoids, polysaccharides and pigments all have the function of removing nitrite[15]. Nitrite removal from chestnut flower extract may be related to the high content of flavonoids in the extract. The specific clearance reasons need to be further studied.

Acknowledgments
We thank the Teaching Group of Food Chemistry, Faculty of Food Science and Engineering of Beijing University of Agriculture for laboratory assistance. We thank Beijing Innovation Consortium of Swine Research System and Graduation Design of the Practical Training Program for the Cross-Cultivation of High-Level Talents in Beijing Colleges and Universities for support. This work was supported by Lan Yanping from Chinese Academy of Agricultural Sciences. Molecular analyses were supported by Beijing Key Laboratory of Agricultural, Product Detection and Control of Spoilage Organisms and Pesticide Residue.
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