Effect of ethanol extract from chestnut flower on nitrite content in pickles

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Abstract. In this paper, chestnut flowers from the Huairou District of Beijing were used as experimental materials. It was aimed to explore the removal effect of nitrite by ethanol extract simulation system of chestnut flower and its impact on nitrite in food. The content of nitrite was measured by the naphthalene ethylenediamine hydrochloride method. In the experiment, the ethanol extract of chestnut flower was added when pickling vegetables. During pickling, adding the ethanol extract of chestnut flower could decrease the content of nitrite. As a consequence, the peak of nitrite was delayed one day due to the addition of 0.2 g/mL ethanol extracting from the chestnut flower.

1. Introduction
Pickle is a vegetable product made by fermentation with lactic acid bacteria. Pickles contain a large number of lactobacillus and lactobacillus metabolites, which have health functions such as regulating intestinal micro ecological balance and improving body immunity, so they are popular with the public. However, during the processing and storage of pickles, the excessive nitrite content will bring potential safety problems to the product [1]. The development process of pickles is mainly lactic acid bacteria fermentation, but the vegetables contain Escherichia coli, Para-Escherichia coli and other harmful bacteria can make the nitrate in vegetables reduced to nitrite. Sodium nitrite is an inorganic compound with the chemical formula shown as a white to yellowish crystalline powder that is extremely soluble in water and hygroscopic, and is best known for its use in processed meat [2]. In the production process of meat products, nitrite has the function of chromogenic [3], and nitrite is used as an irreplaceable additive in the field of meat products [4]. Nitrite accumulates during plant fermentation, because it can be assimilated into plant proteins, but excessive use of nitrogen fertilizer will lead to the accumulation of large amounts of nitrate in plants [5]. At the same time, nitrate in plants can be reduced to nitrite by nitrate reductase. Because vegetables are easy to carry Enterobacteria and Diphtheria bacillus, a large amount of nitrate reductase will be generated in the fermentation process of vegetables, which can reduce nitrate to nitrite and accumulate [6-8]. Nitrite is a strong oxidant, in the human body will react with hemoglobin, so that the human blood can not transport oxygen, the human body due to hypoxia leads to poisoning, and can even lead to death [9]. Studies show that the human body intake of nitrite content in 0.3g-0.5g, will cause human toxic reaction; excessive amounts of 3g can cause death in
humans. Therefore, it is of positive guiding significance for the food safety of pickles to investigate the production law of nitrite in the fermentation process of pickles [10].

Recorded in Compendium of Materia Medica chestnut flowers can be used to treat throat swelling, and recorded according to the Chinese medicine dictionary, chestnut flower can be used to heal the hematochezia, diarrhea, ease children dyspepsia symptoms [11]. In the process of chestnut production leaves a lot of chestnut male flowers, especially in recent years, as the chestnut planting area expands unceasingly, every year to produce more chestnuts. And most of the chestnut flower directly discarded as waste, so that the research and development of chestnut flower has important social significance and economic benefits. Chestnut flowers contain fatty acids, flavonoids, estradiol and protein and other substances. Studies have shown that flavonoids have strong antioxidant activity and can effectively remove nitrites. At present, the main methods are organic solvent extraction, microwave-assisted extraction and water extraction. In this chapter, ethanol will be used to extract chestnut flowers to obtain chestnut flower ethanol extract. The extraction process of chestnut flower ethanol extract was optimized by single factor and orthogonal experiments, and the optimized results were verified. The chemical and physical methods were used to analyze the ethanol extract of chestnut flowers. The effects of ethanol extract from chestnut flower on the content of nitrite in pickling process and pickling time were studied.

2. Materials and Methods

2.1 Materials and reagents
Chestnut flowers were collected from Huairou District in Beijing. All reagents were of the chemical reagents.

2.2 The extraction of the ethanol extract from chestnut flowers
Chestnut flowers froze drying about 48h. Used balance accurately according to the concentration of 1.00 g chestnut flower and added 50 mL of 40% ethanol. Used a glass rod for even mixing. The mixture put into the centrifuge tube and used plastic wrap to seal, then covered the lid to seal. Used ultrasonic cleaner to shake for 30 min at 80 HZ, then used water bath at 40°C for 1.5 h. Took out, used a separatory funnel to filter, then the filtering of ethanol extract from chestnut flowers would been collected in the avoid light place to frozen [12][13].

2.3 The process of making pickle [14]
In the pickle pickling process, 500g radishes were added to the pickle jar, 350mL water with a salt concentration of 4% was added, 20mL chestnut flower ethanol extract was added, 10g sugar was added, 10mL spirit was added, and finally water was added to seal the jar. The sealed pickle jar was placed in a constant temperature incubator for fermentation at 30°C.

2.4 Determination of nitrite content
According to nitrite detection method from Wang [15].

2.5 Statistical methods
The test results were processed by Microsoft Excel software and statistically analyzed by SPSS22.0 software. The results of each sample were repeated three times and expressed as mean ± standard deviation.

3. Results and analysis

3.1 Standard curve of nitrite
The standard curve of nitrite was shown in Figure 1, and the regression equation was y=0.0324x+0.0045, R²=0.999.
3.2 Effect of chestnut flower ethanol extract on nitrite formation during pickle curing

It could be seen from Figure 2 that the change of nitrite in the pickling process was a process of first increasing and then decreasing. It could be seen that the nitrite peak in the blank group appeared on the second day, while the nitrite peak in the chestnut flower ethanol extract group appeared on the third day. The results indicated that the addition of 0.2g/mL chestnut flower ethanol extract could significantly inhibit the formation of nitrite and delay the time of nitrite peak. It could be seen that when pickled at 30°C, the nitrite content basically did not change on the fourth day, and it is safe to eat.

3.3 Effect of addition amount of chestnut flower ethanol extract on nitrite in pickles

As could be seen from Figure 3, the peak value of nitrite added with chestnut flower ethanol extract was always lower than that of nitrite in the blank group. With the addition of chestnut flower ethanol extract increased, the nitrite content in pickle decreased, and the time of nitrite peak appeared was prolonged. The occurrence of nitrite peak delay was consistent with the results of Wang LX [16] on the nitrite change in natural fermentation of Chinese prickly ash for pickles. The chestnut flower ethanol extract had a certain inhibitory effect on nitrate reduction, so 0.2g/mL chestnut flower ethanol extract would delay the production time of nitrite in pickle. The ethanol extract from chestnut flower can effectively reduce the content of nitrite. After salting, the nitrite content of the chestnut flower ethanol extract group was half of the blank group, and the nitrite content was significantly reduced.
4. Conclusion
In this experiment, the ethanol extract of chestnut flower was applied to the actual food production process. Adding chestnut flower ethanol extract in pickling process could inhibit the formation of nitrite in pickles. 0.2g/mL chestnut flower ethanol extract could delay the occurrence time of nitrite peak by one day. The content of nitrite in pickles with chestnut flower ethanol extract was lower after pickling. The nitrite content was half that of the blank group. The nitrite content did not change after being pickled at 30°C for four days.

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