Shandong Special Product—Optimization of Phytic Acid Extraction Technology from Weixian Radish and Antioxidant Activity Research

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Abstract: In this experiment, Weixian radish, a specialty of Shandong, was used as raw material, and single factor and orthogonal test methods were used. The extraction process conditions of phytic acid were studied and optimized, and its antioxidant activity was studied. The results of the study showed that the phytic acid in Weixian radish was the optimal condition for the extraction of phytic acid from Weixian radish at the extraction time of 30 minutes, temperature of 25°C, and material-to-liquid ratio of 1:55, and had the greatest impact on the extraction of phytic acid is the temperature. The second was the material-to-liquid ratio, and finally the extraction time. The content of phytic acid extracted under optimal conditions was 0.1276mg/g. The scavenging rate of phytic acid extract under the optimal conditions to DPPH free radical was 30.63%. The ·OH radical scavenging rate was 83.12%, the FRAP value was 3.853μmol/L, and the antioxidant effect was significant.

1. Introduction
Weixian radish is a famous variety of radish in Shandong Province. It is named after it was originally produced in Weixian, Shandong. It has a history of more than 300 years of cultivation. The skin color of Weixian radish is dark green, the flesh is emerald green, the taste is spicy, sweet, crisp, and the taste is delicious. It has a strong and unique local flavor and distinctive regional characteristics. Weixian radish is rich in nutrition and can be called a health food [1]. According to tests, the fleshy roots of Weixian radish contain a variety of vitamins, mineral elements and active substances that are necessary to maintain human metabolism. Weixian radish contains amylase, so it has the effect of curing stomach distension, cough and phlegm [2]. The latest research shows that Weixian radish contains lignin [3], which can increase the vitality of human macrophages by 2 to 3 times, improve human immunity and prevent cancer. It also contains an enzyme that can decompose ammonium nitrite, which can reduce the carcinogenic effects of nitrite. Weixian radish is a health food that integrates food nutritional value and medicinal value. It has extremely high nutritional value and research value [4].

Phytic acid is also called creatine, cyclohexanol phosphate, it is mainly found in the seeds, roots and stems of plants [5]. Phytic acid is widely used in the chemical, petroleum, metallurgical, and daily chemical industries. It can be used as an antioxidant for fats and oils, a preservative for food and fruits, an anti-sticking agent for polyvinyl chloride polymerizers, etc., especially as one of the important raw materials for the production of inositol [6].
2. Materials and methods

2.1. Materials and equipment

2.1.1. Raw materials and main reagents. Weixian radish is a variety of Gaojiaoqing, purchased at Weifang Farmers Market. DPPH (Tokyo Chemical Industry Co., Ltd.); TPTZ (Shanghai Eka Biotechnology Co., Ltd.); Sodium Phytate Standards (Laiyang Wanjiewei Biological Engineering Co., Ltd.); Sulfosalicylic Acid (Shanghai Lingjin Fine Chemical Co., Ltd.).

2.1.2. Main test instruments and equipment. 750T multifunctional grinder (Boou Hardware Factory); 80-2 electric centrifuge (Shanghai Meixiang Instrument Co., Ltd.); T502E precision electronic balance (Shanghai Meixiang Instrument Co., Ltd.); 722N visible light spectrophotometer (Shanghai Jinghua Technology Instrument Co., Ltd.).

2.2. Test method

2.2.1. The extraction process and detection method of phytic acid in Weixian radish. It can take fresh Weixian radish, dry, crush, sieved, add a certain proportion of 0.1 mol/L HCl solution, and put it in a constant temperature water bath for extraction for about 30 minutes. Centrifuge at 3000r/min for 15 minutes, take the supernatant and dilute to 100 mL with distilled water. It can add 2 drops of 10% sulfosalicylic acid solution, then be titrated with standard ferric chloride solution until the lavender color does not fade, and calculate the phytic acid content according to the following formula[7].

\[ \text{Phytic acid content (mg/g)} = 20C \times V \times 0.2357 \times 10/W, \]

where C is the concentration of ferric chloride solution (mol/L), and V is the volume of ferric chloride consumed by titration (mL), W is the dry basis weight of the sample (g).

2.2.2. Single factor experimental design. It can weigh 1.00 g of Weixian radish dry powder, and add a hydrochloric acid solution with a concentration of 0.1 mol/L according to the ratio of material to liquid. For material-to-liquid ratio (1:20, 1:30, 1:40, 1:50, 1:60), extraction temperature (20°C, 30°C, 40°C, 50°C, 60°C), extraction time (10 mins, 20 mins, 30 mins, 40 mins, 50 mins) and other factors for single factor test. After centrifugation, the supernatant is taken to a constant volume, and the phytic acid content in the extract is determined.

2.2.3. Orthogonal test determines the best extraction conditions of phytic acid in Weixian radish. On the basis of single factor experiment, according to the optimal factors, the extraction temperature (A), material-to-liquid ratio (B), extraction time (C) and other process conditions are subjected to a three-factor three-level orthogonal test, and the L9 (3^3) orthogonal table is selected. The three factors are comprehensively investigated to determine the best extraction process for phytic acid in Weixian radish.

2.2.4. Antioxidant activity test. The phytic acid is extracted under the optimal extraction process conditions, and the volume is adjusted to 100 mL with distilled water after centrifugation to prepare the phytic acid extract. FRAP value[8], DPPH clearance rate[9] and ·OH clearance rate[10] are measured on the extract.
3. Results and analysis

3.1. Analysis of single factor test results

3.1.1. The effect of material-to-liquid ratio on the extraction rate of phytic acid in Weixian radish.

![Figure 1. Effect of material-liquid ratio on extraction rate of phytic acid](image1)

It can be seen from Figure 1 that the extraction rate of phytic acid increased with the increase of the material-to-liquid ratio. In the experiment, it was analyzed that if the amount of hydrochloric acid solution added was too small, the combination of phytic acid and other substances could not be completely destroyed, and phytic acid could not be fully analyzed. As a result, the extraction rate of phytic acid was reduced. After the material-to-liquid ratio reaches 1:50, the increase in the extraction of phytic acid was not obvious. So a material-to-liquid ratio of 1:50 was selected as the better material-liquid ratio.

3.1.2. The effect of extraction temperature on the extraction rate of phytic acid in Weixian radish.

![Figure 2. Effect of extraction temperature on extraction rate of phytic acid](image2)

It can be seen from figure 2 that the extraction rate of phytic acid first rose and then declined with the increase of the temperature of the water bath. When the temperature was low, the extraction rate of phytic acid increased with the increase of temperature, and reached the maximum when the temperature of the water bath was 30°C. Analysis believes that increasing the temperature will increase the molecular kinetic energy, which is conducive to precipitation. However, if the temperature is too high, it will cause the product to be damaged or combined sedimentation, which will reduce the extraction of phytic acid. Therefore, 30°C was selected as the optimal extraction temperature.
3.1.3. Effect of extraction time on extraction rate of phytic acid from radish in Weixian

It can be seen from Figure 3 that the extraction of phytic acid had an upward trend as the extraction time increases. Among them, the increase was more obvious in the time period of 10~30 mins, and the downward trend was shown in the time period of 30~50 mins. The extension of the extraction time will help the dissolution of phytic acid. When the product was basically dissolved, the extension of the time will not continue to increase the yield, but will cause losses due to prolonged contact with oxygen in the air for a long time, so the extraction time was 30 mins is the best time for phytic acid extraction.

3.2. Optimal extraction conditions of phytic acid from radish in Weixian County were determined by orthogonal test

According to the results of single factor test, the main influencing factors were investigated by using L₉(3³) orthogonal table, which is shown in Table 1.

| Level | A extraction temperature (°C) | B ratio of feed to liquid (g/mL) | C extraction time(min) |
|-------|-------------------------------|---------------------------------|------------------------|
| 1     | 25                            | 1:45                            | 25                     |
| 2     | 30                            | 1:50                            | 30                     |
| 3     | 35                            | 1:55                            | 35                     |

The results of the orthogonal test were shown in table 2. According to the magnitude of the extreme difference, the primary and secondary order of the extraction of phytic acid from Weixian radish was A>B>C. That meant, the most important factor affecting the extraction of phytic acid was the extraction temperature, followed by the ratio of material-to-liquid, and finally the extraction time. According to the average value, the optimal level was selected as A₁B₃C₂, that meant, the extraction conditions was the extraction temperature of 25°C, the material-to-liquid ratio of 1:55, and the extraction temperature 30 mins. Because this condition was not in the experimental design, a verification test was required. The verification test tested that the content of phytic acid extracted under this condition reached 0.1276 mg/g, which was indeed the best.

| Test number | A | B | C | Extraction rate of phytic acid (mg/g) |
|-------------|---|---|---|--------------------------------------|
| 1           | 1 | 1 | 1 | 0.0906                                |
3.2.1. Determination of phytic acid’s antioxidant capacity

The phytic acid was extracted under the optimal extraction conditions. The extraction liquid was subjected to the determination of the ·OH clearance rate, DPPH clearance rate and FRAP value. The test results were shown in table 3 below. After testing, under the best process conditions, the phytic acid extract had a 30.63% removal rate of ·OH, a 83.12% of DPPH removal rate, and a 3.853μmol/L of FRAP value, which had strong antioxidant capacity.

|       | ·OH determination of clearance rate (%) | DPPH determination of clearance rate (%) | FRAP(μmol/L) |
|-------|----------------------------------------|------------------------------------------|--------------|
| Weixian radish | 30.63                                   | 83.12                                    | 3.853        |

4. Conclusion

In this study, weixian radish was used as raw material to study the extraction process of phytic acid contained in it. The results showed that the most significant influence on the extraction process of phytic acid in Weixian radish is the extraction temperature, followed by the ratio of material to liquid, and finally the extraction time. The strongest extraction conditions for extracting phytic acid from Weixian radish were temperature 25℃, material-to-liquid ratio 1:55, and time 30min. The phytic acid content of Weixian radish extracted under this condition is 0.1276 mg/g, which was the highest. Under the optimal conditions, the phytic acid extract had a scavenging rate of 30.63% for DPPH free radicals, a scavenging rate for ·OH free radicals of 83.12%, and a FRAP value of 3.853 μmol/L. The antioxidant effect was significant.

Due to its spicy taste, Weixian radish has limited its sales and development to a large extent. Therefore, weixian radish can be combined with the characteristics of high yield and in-depth processing to carry out related research on the extraction of phytic acid and antioxidant properties. It is conducive to expanding the processing and utilization of Weixian radish.

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