Study on chemical properties of chestnut shell pigment

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Abstract: In this thesis, the chemical properties of chestnut shell pigments were mainly studied. Through a series of pigment stability experiments, it could be learned that the pigment of chestnut shell was sensitive to different pH and heat, stable to light, and had certain tolerance to oxidant H₂O₂ and reducing agent ascorbic acid. Mn²⁺ and Zn²⁺ had certain enhancement effects on color of the pigments. Cu²⁺, Fe²⁺, Fe³⁺ and Al³⁺ could destroy the stability of pigment, and caused precipitate. The pigment was also stable to the commonly used as food additives.

1. Introduction
Chestnut shell is a kind of chestnut plant of fagaceae. It has been planted in China for more than 3,000 years. In recent years, the total yield of Chinese chestnut has been the highest in the world[1-2]. The production of chestnut pulp has been quite large. However, chestnut shells have not been effectively utilized as processing waste[3]. Generally speaking, most of the components of chestnut were lignin, cellulose, hemicellulose and other substances. A series of studies have shown that the chestnut shell contains phenols, organic acids, sugars, polysaccharides (or glycosides), flavonoids (or saponins), phytosterols (or triterpenoids), lactones, coumarins (or their glycosides) and tannins[4-5]. Among the known chemical constituents of chestnut shell, organic acids and their derivatives are the most abundant: P-hydroxybenzoic acid, gallic acid, 3, 4-dihydroxybenzoic acid, gentian acid, salicylic acid, syringic acid, chlorogenic acid. Sterols: beta-sitosterol, beta-sitosterol-3-O-beta-D-glucoside, stigmasterol-3-en-3beta, 7a-ol, ergosterol-6,22-diene-3beta, 5a, 8a-triol, stigmasterol-4-en-6-ol, stigmasterol-4-en-6beta-ol-3-one, stigmasterol-5-en-3beta-hydroxy-7-one[6-9]. Flavonoids compounds are rutin, apigenin, kaempferol, quercetin and forsythiaside[10]. Terpenoids and their derivatives are: Oleanolic acid, betulinic acid, ursolic acid[11]. Other compounds include beta-carotene, methyl toluene 2, 6-diaminocarbamate, methyl p-hydroxy cinnamate, vanillin and lactose[12]. The development and utilization of new natural pigments have became the current research trend. Among many natural pigments, brown pigments played an important role in food coloring and were widely used, but there are not many varieties at present[13]. Castanea mollissima shell was a waste in the process of chestnut processing, from which a natural brown pigment could be extracted. This brown pigment has high...
content, strong dyeing power, and has certain antioxidant and bacteriostasis, so it has a broad development prospects[14]. In this study, the chemical property and stability of chestnut shell pigments were analyzed to evaluate the applicability and safety of pigments.

2. Materials and reagents

2.1. Materials
Chestnut shell was provided by Shandong processing plant.

2.2. Reagents
Ethyl acetate was purchased from Beijing chemical works, methanol, acetone and Sephadex LH-20 were purchased from Tianjin Kermeel chemical reagent development center.

3. Experimental methods

3.1. Study on the stability of chestnut shell pigment

3.1.1. Effect of pH on the stability of pigment
The buffer solution with pH of 3.0, 5.0, 7.0, 9.16 and 10.83 were prepared. Then taken 1 mL 0.5% pigmentation solution. It was volumetrized to 25 mL with the prepared buffer solution, and placed at ordinary temperature for 1 h. The absorbance was measured at 400 nm. At the same time, the color change of the pigmentation solution was observed and recorded[15].

3.1.2. Effect of temperature on the stability of pigments
25 mL 0.02% pigmentation solution was placed in a thermostatic waterbath at 20, 40, 60, 80 and 100 ℃ for 0.5, 1, 1.5 and 2 h. The solution was rapidly cooled to ordinary temperature. The color change of the solution was observed and the absorbance was measured at 400 nm[16].

3.1.3. Effect of light on the stability of pigments
50 mL 0.02% pigments solution was taken in transparent tube, placed one group in the dark and another group in the natural light for 6 days, observed the color change of the solution every day and measured the absorbance at 400 nm[17].

3.1.4. Effects of oxidants and reductants on the stability of pigments
1 mL 0.5% pigment aqueous solution was added with different concentrations of H2O2 and ascorbic acid respectively. The concentration of H2O2 or ascorbic acid in the system was 0, 0.05, 0.01, 0.5 and 1%, respectively, with a buffer solution of pH 7.0 to 25 mL. After 30 minutes of sheltering, absorbance was measured at 400 nm.

3.1.5. Effect of metal ions on the stability of pigments
Metal ion solutions (K+, Na+, Ca2+, Fe2+, Fe3+, Al3+, Zn2+, Mn2+, Cu2+) with 2 mmol/L concentration were prepared respectively. 1 mL 0.5% pigmentation solution was used to fix the volume of the above metal ion solution to 25 mL. The pigmentation solution was kept away from light for 2 h and 24 h at ordinary temperature. The change of the pigmentation solution was observed and the absorbance was determined at 400 nm[18].

3.1.6. Effect of some food additives on the stability of pigments
According to the designed concentration, 10% sucrose solution, 0.2% sodium benzoate solution, 0.2% potassium sorbate solution and 0.5% sodium chloride solution were prepared. 1 mL 0.5% pigmentation solution was used to determine the absorbance of the above additive solutions at 400 nm.
4. Results and discussions

4.1. Effect of pH on the stability of pigment

Table 1. Effects of pH on the stability of the pigment

| pH   | 3.0       | 5.0       | 7.0       | 9.16      | 10.83     |
|------|-----------|-----------|-----------|-----------|-----------|
| Abs  | 0.546±0.011 | 0.521±0.006 | 0.601±0.007 | 0.766±0.011 | 0.974±0.018 |
| Color| yellow    | yellow    | brown     | reddish brown | reddish brown |

It could be seen from the Table 1, the pigment solution was stable at pH 3 and 5, and the color of the solution was unstable in alkaline environment.

4.2. Effect of temperature on the stability of pigments

Figure 1. Effects of temperature and heating time on the stability of the pigment

From the results of Fig 1, it can be seen that with the increase of heating temperature, the absorbance of the pigment solution gradually increased, and the color of the solution gradually deepened. It shown that the pigment was unstable to heat, and a short time of heating could make the pigments producing new dark substances and changing their colors.

4.3. The effect of light on the stability of pigments

Figure 2. Effects of light on the stability of the pigment

The absorbance of chestnut shell pigments solution irradiated by natural light for 6 days was not significantly different from that of the pigments solution preserved in dark (p > 0.05), and the color of chestnut shell pigments solution was also not different from that of the pigments solution preserved in dark light (p > 0.05). It could be concluded that the pigment of chestnut shell was stable to light.
4.4. Effects of oxidants and reductants on the stability of pigments

After treatment with different concentrations of H₂O₂ and ascorbic acid, the color change of chestnut shell pigment solution was not obvious. The brown pigment of chestnut shell had certain tolerance to oxidant H₂O₂ and reducing agent ascorbic acid.

4.5. Effect of metal ions on the stability of pigments

Mn²⁺ and Zn²⁺ had certain enhancement effects on color of the pigments. Cu²⁺, Fe²⁺, Fe³⁺ and Al³⁺ could destroy the stability of pigment, and caused precipitate. The pigment was also stable to the commonly used as food additives.

4.6. Effect of some food additives on the stability of pigments

After adding food additives sucrose, sodium benzoate and NaCl to the solution for 24 hours, the
absorbance of the solution had no significant difference compared with the control group (P > 0.05), indicating that it had no effect on the stability of the pigment. After adding sorbate, the absorbance of the solution changed significantly (p < 0.01) after 24h, probably because the solution of sorbate was weakly alkaline, which changed the pH of the system and absorbance of the solution.

5. Conclusion
A series of stability tests showed that chestnut shell pigments were sensitive to pH and heat, stable to light, and tolerant to oxidant H₂O₂ and reducing agent ascorbic acid. For K⁺, Na⁺, Ca²⁺ stabilization, Mn²⁺ and Zn²⁺ have certain chromogenic effect on pigments. Cu²⁺, Fe²⁺, Fe³⁺, Al³⁺ can destroy their stability and make pigments precipitate. Pigments are also stable to commonly used food additives.

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