Characteristic of the Pigments Extracted by Alkali from Chestnut Shell

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Abstract. Sampled with the pigments extracted from chestnut shell, the characteristic was compared with caramel pigment. The spectrophotometer was used to measure the absorbance at different wavelengths. The chromaticness, red index and the stability of the pigments from the chestnut shell related. Compared with caramel pigments, lower chromacticness and higher red index of shell pigments were shown. Furthermore, the chromaticness and red index of samples were increased with the pH value. In addition, the stability of the pigments from chestnut shell was better when the pH value was between 3.5~6.0. But its stability in salt solution was not as good as caramel pigments.

1. Introduction

Food pigments can be classified as synthetic and natural pigments. Due to the risk of synthetic pigments on human health, the development and utilization of new natural pigments are concerned in food industry currently [1-2]. Natural brown pigment can be extracted from chestnut shell which is the waste in chestnut production. This brown pigments’ content is substantial in shell, and its characteristics are steady [3-4]. Most researchers found that the main components of the pigments are flavonoids. Flavonoids generally have antioxidant activity and bacteriostatic effect; therefore, the chestnut shell pigments have not only coloring function, but also the certain medicinal value [5-7]. In short, the pigments have a promising development prospect [8]. According to the different solutions, the measures of pigment extraction are usually divided into alcohol-extraction and alkali-extraction. The research shown that the alkali-extracted pigment was superior to the alcohol-extracted pigment in terms of extraction rate and antioxidation [9]. However, there were few studies on the characteristics of the alkali-extracted chestnut shell pigments. In this paper, the chromaticness, red index and stability of the alkali-extracted chestnut shell pigments were analyzed in order to provide the basis for utilization of the pigments.

2. Materials and methods

2.1. Samples and chemicals

Pigments were extracted by sodium hydrogen carbonate from chestnut shell. Caramel pigments were purchased from Beijing Xiaguang Additives Shop. All other reagents used in this experiment were of analytical grade.

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2.2. Determination of chromaticness [10]
Caramel pigments were the most common natural brown pigments, so the characteristics of chestnut shell pigments were compared with the caramel pigments. 0.1% (W/V) shell and caramel pigment solutions were prepared separately. The absorbance of pigment solutions (OD1) were measured respectively at 610 nm with a spectrophotometer. And the measurement was repeated several times to take the average values. The chromaticness was estimated by the following formula:

\[ \text{chromaticness (EBC unit)} = \frac{\text{OD}_1 \times 20000}{0.076} \]  

(1)

2.3. Determination of red index [11]
The absorbance of 0.1% (W/V) pigment solutions (OD2) was read at 510 nm with a spectrophotometer. The red index was estimated by the following formula:

\[ \text{red index} = 10 \times \log \frac{\text{OD}_1}{\text{OD}_2} \]  

(2)

2.4. Study on the relationship between chromaticness, red index and pH value [12]
1% (W/V) shell and caramel pigment solutions were prepared separately. Then 10 mL of the pigment solutions were moved in a 100mL volumetric flask by graduated pipette respectively, and five volumetric flasks were used for each pigment solution. A certain concentration of hydrochloric acid or sodium hydroxide solution was added into each flask to make a gradient of the pH value of 0.1% pigment solutions. The solutions were diluted to 100 mL with distilled water. The pH values of the diluted pigment solutions were measured with an acid meter. Then the absorbances of the solutions at 510 nm and 610 nm were measured with a spectrophotometer.

2.5. Effect of the pH value on pigment stability [13]
0.5% (W/V) chestnut shell and caramel pigment solutions were added in 25 mL colorimetric tubes separately. Then 20 mL of acetate buffer solutions with pH 3.5, 4.0, 4.5, 5.0, 5.5 or 6.0 were added in the tubes respectively. After 5 days' standing, the effects of different pH values on stability of the pigment solutions were estimated by the generated time and amount of the turbidity.

2.6. Study on salt tolerance [14]
0.5% (W/V) chestnut shell and caramel pigment solutions were added in 25 mL colorimetric tubes separately. Then 20 mL of sodium chloride solutions at concentration of 0%, 2%, 3%, 4%, 5% or 6% were added in the tubes respectively. After 5 days' standing, the effects of different concentrations of sodium chloride solution on stability of the pigment solutions were estimated by the generated time and amount of the turbidity.

3. Results and discussions
From table 1, the chromaticness of caramel pigment was greater than that of the alkali-extracted chestnut shell pigment, but the red index of chestnut shell pigment was higher. According to the sensory observation, the color of caramel pigment solution was brownish red and darker than that of chestnut shell pigment solution. The hue of shell pigment solution was closer to redness.

The chromaticness of pigment was an indicator of the different color shades, and it was expressed in EBC units. 20000 EBC indicated that the optical density of pigment was 0.076 at 610nm. For the same sample, the optical density increased because of darkening the color of solution. It can be seen from formula (1), the chromaticness was proportional to the optical density. Therefore, larger chromaticness of pigments reflected darker color of pigment solutions\textsuperscript{[15]}. However, it was different for various samples. The compositions of caramel and chestnut shell pigments were different. Caramel pigment was a mixture of various sugars by dehydration and condensation\textsuperscript{[16]}, and the main coloring substance of chestnut shell pigment was flavonoids. The chromaticness of two kinds of pigments measured at 610 nm had a very large difference because the optical density was greatly affected by the solutes.
Red index indicated the strength of redness as the main color [15]. The red index of chestnut shell pigment was larger than that of caramel pigment, which meant the hue of shell pigment was closer to the redness.

Table 1. Chromaticness and red index of various pigments

| Pigments                     | Caramel pigment | Chestnut shell pigment |
|------------------------------|-----------------|------------------------|
| Chromaticness (EBC)          | 161052±254      | 33421±183              |
| Red index                    | 3.91±0.86       | 5.89±0.32              |

*Results expressed as average ± standard deviation of the analyzed parameters.

Figure 1 and 2 shown that the relationship between the chromaticness of the caramel pigment and pH value belonged to the quadratic function, the same to the red index. When the pH value was between 3 and 4, the chromaticness had a minimum value and the red index had a maximum value. When the pH value was higher than 4, the chromaticness was increasing and the red index was reducing with the increase of the pH value.

It can be seen from figure 3 and 4; the chromaticness of the alkali-extracted chestnut shell pigment was increasing with the increase of pH value in general. From the sensory observation, the high pH values darkened the color of the pigment solutions. On the other hand, the red index was proportional to the pH value in general. But it was decreasing when the pH value was between 6 and 8.

Caramel pigment was a non-single mixture (about 100 different compounds) produced by caramelization reactions [16]. Due to the complexity of the chromophores, the effects of the pH value on the chromaticness and red index of caramel pigment were different from those of chestnut shell pigments. The main coloring substances of chestnut shell pigment were flavonoids with phenolic hydroxyl groups [17]. The phenolic hydroxyl groups possessed weak acidity, and the alkaline environment was conducive to its extraction. Thus, the high pH values were conducive to darkening the color of the pigments.
From table 2, the alkali-extracted chestnut shell pigment had excellent stability when the pH value was between 3.5~6.0. The caramel pigment also shown good stability at pH 3.5~5.0. However, when the pH value rose to 5.0~6.0, a small amount of turbidity tended to appear after 3 days’ standing.

The caramel pigment was colloidal solution with electric charge. The caramel pigment by ammonia process carried positive charge with an isoelectric point of about 6.0 [18]. The macromolecules colloidal solution was the most unstable close to isoelectric point and it was liable to form deposition. Therefore, the caramel pigment solutions appeared turbid near the isoelectric point place. The chestnut shell pigment was not colloid so it was more stable than caramel pigment.

**Table 2. Effect of the pH value on pigment stability**

| Pigments                | pH value | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|-------------------------|----------|-------|-------|-------|-------|-------|
| Caramel pigment         | 3.5      | -     | -     | -     | -     | -     |
| Caramel pigment         | 4.0      | -     | -     | -     | -     | -     |
| Caramel pigment         | 4.5      | -     | -     | -     | -     | -     |
| Caramel pigment         | 5.0      | -     | -     | -     | -     | -     |
| Caramel pigment         | 5.5      | -     | -     | -     | +     | +     |
| Caramel pigment         | 6.0      | -     | -     | +     | +     | +     |
| Chestnut shell pigment  | 3.5      | -     | -     | -     | -     | -     |
| Chestnut shell pigment  | 4.0      | -     | -     | -     | -     | -     |
| Chestnut shell pigment  | 4.5      | -     | -     | -     | -     | -     |
| Chestnut shell pigment  | 5.0      | -     | -     | -     | -     | -     |
| Chestnut shell pigment  | 5.5      | -     | -     | -     | -     | -     |
| Chestnut shell pigment  | 6.0      | -     | -     | -     | -     | -     |

"*" means steady; "+" means that a small amount of turbidity appears in the solution; "++" means that about half of the solution becomes turbid; "+++" means that a large amount of turbidity appears in the solution.

According to table 3, different concentrations of sodium chloride solutions impacted on pigment stability. The caramel pigment had great stability in sodium chloride solutions. However, the stability of the chestnut shell pigment was not as good as caramel in salt solutions. There was a small amount
of turbidity in the solutions at each concentration, and the amount of turbidity increased slightly with
the increase of NaCl concentration and storage time.

This was because that, the caramel pigment by ammonia process possessed good salt tolerance. The pigment was primarily used for coloring soy sauce in the food industry. In the process of extracting chestnut shell pigment by alkali method, the proteins in the chestnut shell were prone to hydrolyze and dissolve as impurity in the hot alkaline environment. The proteins contained in the unpurified pigments were precipitated out in inorganic salt solutions, causing the solutions to become cloudy. And the amount of sediment increased with the increase of concentrations of the inorganic salt.

**Table 3. Effect of NaCl concentration on pigment stability**

| Pigments            | NaCl concentration | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---------------------|--------------------|-------|-------|-------|-------|-------|
| Caramel pigment     | 0%                 | -     | -     | -     | -     | -     |
| Caramel pigment     | 2%                 | -     | -     | -     | +     | +     |
| Caramel pigment     | 3%                 | -     | -     | -     | -     | +     |
| Caramel pigment     | 4%                 | -     | -     | -     | -     | -     |
| Caramel pigment     | 5%                 | -     | -     | -     | -     | -     |
| Caramel pigment     | 6%                 | -     | -     | -     | -     | -     |
| Chestnut shell pigment | 0%         | -     | -     | -     | -     | -     |
| Chestnut shell pigment | 2%       | +     | +     | +     | +     | +     |
| Chestnut shell pigment | 3%       | +     | +     | +     | +     | +     |
| Chestnut shell pigment | 4%       | +     | +     | +     | +     | +     |
| Chestnut shell pigment | 5%       | +     | +     | +     | ++    | ++    |
| Chestnut shell pigment | 6%       | +     | +     | +     | ++    | ++    |

*"-" means steady; "+" means that a small amount of turbidity appears in the solution; "++" means that about half of the solution becomes turbid; "+++" means that a large amount of turbidity appears in the solution.

4. Conclusions

Higher red index and lower chromaticness of the pigments extracted by alkali solution from chestnut shell were shown compared with caramel pigment. The effects of the pH value on the chromaticness and the red index of shell pigments reflected that rising the pH value was conducive to darkening the color and presenting redness. The stability of chestnut shell pigment was better than that of caramel pigment when the pH value was between 3.5~6.0. However, the salt tolerance of chestnut shell pigment was not as good as caramel pigment.

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6. References

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