Study on extraction of anthocyanin from pericarp waste by SDS precipitation

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Abstract. In recent years, our country (China) has invested more and more in environmental protection, and resource recycling concept is also gaining in popularity. The free throw of the peel wastes both increases the load on the environment and wastes the useful ingredients in the peel. It is a natural pigment anthocyanin, not only has the natural colorants function but also has a high biological value and medicinal value. Useful anthocyanin extracted from the peel waste is a manifestation of the principle of "turning waste into treasure and utilization". The Surfactant precipitation technique is a relatively new plant anthocyanin extraction method. Anionic surfactant-sodium dodecyl sulfate was selected to experiment, and the extraction efficiency of sodium dodecyl sulfate precipitation technique peel waste anthocyanin was discussed. Parameters such as surfactants, potassium chloride, sodium chloride concentration pH, and other factors on the recovery of the solvent category extraction affect the results. The results showed that optimal experimental conditions for 3mL crude extract solution, as follows: pH is 2, using 4mL5mmol/L of SDS, 1.5mL 6mmol/L of KCl, 1mL AR acetone, 1mL 0.25mol/L of NaCl, under the best condition, the rate of anthocyanin extraction was up to 60.81%.

1.Introduction

Anthocyanins are also called anthocyanins. It is a natural, water-soluble pigment belonging to the flavonoid polyphenolic compound[1]. Flavonoids have the structure of 2-phenyl benzofuran and 2-phenylbenzopyrone. The anthocyanin pigment having the former structure is an element of anthocyanidin. In our daily life, we can see various plants in nature. These pigments are commonly found in flowers, fruits, stems and seeds. Its basic structure is 3,5,7-trihydroxy-2-phenyl benzopyran[2].

Anthocyanin is a strong coloring agent and has a strong coloring function, but it also has a variety of important physiological functions and medicinal functions, such as beauty and beauty, anti-aging[3] anti-cancer[4-6], lipid-lowering diet, and improving vision[7]. It can be applied to food, medical drugs, cosmetics and other fields[1,8]. China is a large country with a large population. Whether it is in the food industry or the pharmaceutical industry and the cosmetics industry, consumption is huge. Therefore, Studying the extraction of anthocyanins from plants has great economic significance [9-12]
Although humans have now studied the chemical synthesis and biosynthesis to synthesize cyanin, most products still take the extraction of anthocyanins from natural plants[13-15]. Sodium dodecyl sulfonate (The abbreviation SDS), which is a sulfonate, has a molecular formula of $\text{C}_{12}\text{H}_{25}\text{SO}_{3}\text{Na}$, a molecular weight of 272.38, and the structural formula is as follows [16]:

$$\text{CH}_3\text{(CH}_2)_{10}\text{CH}\text{S}^-\text{ONa}^-\text{O}$$

The molecular structure of SDS is directly linked to the C atom and S atom. It is easily soluble in water and is white powder. SDS is an anionic surfactant[17].

This experiment is first to use hydrochloric acid extraction method to extract crude anthocyanin. Afterward, the surfactant is combined with the anthocyanin to form a composite system and precipitated, and then some recovery reagents are added to decompose the composite system again. Through such a process, the purity of the crude anthocyanin product is increased.

2. Materials and methods

2.1. Experimental materials

Apple peel waste, taken from peel waste in fruit stores.

2.2. Preparation of crude anthocyanins and anthocyanin-surfactant complex system

Accurately weigh 5.00 g of apple peel to squeeze juice, place in a beaker and add 20 mL of water. Add 50 mL of a 0.1 mol/L hydrochloric acid solution. Then, it was transferred to a round-bottomed flask. The reaction was magnetically stirred at 30°C for 4 h at a rotation speed of 20 r/min. Then filter, the filtrate is an anthocyanin crude solution.

A certain amount of sodium dodecyl sulfate (SDS) was added to an aqueous solution containing the crude anthocyanin and potassium chloride. When precipitation occurs in the solution (the anthocyanin-surfactant complex system), shake well and then centrifuge for 15 min. Remove the supernatant, and the remaining was rinsed with distilled water. Centrifuge again, separate and leave a precipitate. Acetone, ethanol, and methanol were respectively added as recovery solvents to the precipitate. When the recovered solvent is added, the precipitate dissolves. Add a small amount of 0.25 mmol/L sodium chloride. The solution precipitated out of the surfactant anthocyanin. The precipitation was complete, the surfactant remained in the organic phase. The precipitated anthocyanin was dissolved in a new aqueous phase, and the absorbance (A) of the product at 281 nm was measured by a spectrophotometer. The purity of the anthocyanin and the extraction rate of the crude anthocyanin were compared. Extraction rate = After extracted solution A/anthocyanin crude A * 100%.

2.3. Preparation of relevant experimental reagent solution

Preparation of 100 mL 0.1 mol/L hydrochloric acid solution: Pipette 0.85 mL of hydrochloric acid with a volume fraction of 36% to 38% and dilute to 100 mL with a 100 mL volumetric flask.

Preparation of 100 mL 7 mmol/L sodium dodecyl sulfate solution: Accurately weigh 0.1907 g of analytically pure SDS powder and add 100 mL of water to dissolve.

Preparation of 100 mL 0.25 mol/L sodium chloride solution: Accurately weigh 1.4610 g analytical sodium chloride powder and add 100 mL water to dissolve.

Preparation of 100 mL 0.2 mol/L potassium chloride solution: Accurately weigh 1.4910 g of analytical potassium chloride powder and add 100 mL of water to dissolve.

If other concentrations of SDS, sodium chloride solution and potassium chloride solution are required, dilute to the desired concentration with the prepared solution.
3. Results and Discussion

3.1. Effect of SDS concentration on extraction of anthocyanin in apple peel

Undoubtedly the choice of sodium dodecyl sulfate concentration is the most important item in this technology since the addition of potassium chloride may change the critical micelle concentration of SDS[18]. When setting this variable, to ensure that the concentration of SDS is lower than CMC (1.24×10⁻² mol/L), this paper selects several ranges of concentration far below the critical micelle concentration of sodium dodecyl sulfate in pure water.

Different quantities of chemical reagent SDS were weighed, and six groups of sodium dodecyl sulfate solutions with different concentrations were respectively configured: 0.002 mol/L, 0.003 mol/L, 0.004 mol/L, 0.005 mol/L, and 0.006 mol/L, each group plus 4 mL SDS solution.

Among them, the dosage of the same pH crude anthocyanin in each group was 3 mL; The amount of KCl solution for 1 mmol/L was 1.5 mL; The amount of acetone with a volume fraction of 5% was 3 mL and the amount of NaCl solution for 0.25 mol/L was 1 mL.

As is shown in Figure. 1, when the concentration of SDS was 4 mmol/L, the extraction rate of anthocyanin was the highest, reaching 16.33%. Surfactant precipitation technology can be seen as a transition between two states. The first state is the present state (anthocyanin and sodium dodecyl sulfate); the second is a new state (an anthocyanin-SDS surfactant complex system). During the experiment, the concentration of SDS was controlled under the CMC (1.24×10⁻² mol/L) to prevent the formation of reverse micelles. From the experimental results, the high or low SDS concentration affects the extraction rate of anthocyanins. The best SDS concentration is 4 mmol/L, so the following experiments use this concentration.

3.2. Effect of SDS dosage on the extraction of anthocyanin from apple peel

Set six groups of 0.004 mol/L SDS for 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 6 mL experiments. Among them, the dosage of the same pH crude anthocyanin in each group was 3 mL; The amount of KCl solution for 1 mmol/L was 1.5 mL; The amount of acetone with a volume fraction of 5% was 3 mL and the amount of NaCl solution for 0.25 mol/L was 1 mL.
Fig. 2. Effect of SDS dosage on the extraction rate of anthocyanin in apple peel.

Analyzing Figure 2, there may be errors in the first data. Judging from the overall trend, it showed a trend of rising first and then decreasing. It can be seen that when the amount of 0.004 mol/L SDS is 4 mL, the extraction rate is the highest, so this amount is the optimal amount for this reaction. The data shows that when the amount of SDS is relatively small, the amount of surfactant is insufficient to be combined with anthocyanin to extract, which directly reduces the extraction rate of anthocyanin; when the amount of SDS is increased, the extraction rate of anthocyanin also increases. However, it is not always showing a rising trend. When the amount exceeds 4 mL, the extraction rate is reduced, indicating that there is an optimum value for the amount of SDS.

3.3. Effect of ionic strength on extraction of anthocyanin in apple peel

Six groups of KCl solutions with different concentrations were prepared: 2mmol/L, 4mmol/L, 6mmol/L, 8mmol/L, 10mmol/L, 12mmol/L. Each group plus 4mL SDS solution. Among them, the dosage of the same pH crude anthocyanin in each group was 3mL; The amount of acetone with a volume fraction of 5% was 3mL and the amount of NaCl solution for 0.25mol/L was 1mL.

Fig. 3. Effect of ionic strength on the extraction rate of anthocyanin in apple peel.

In the entire reaction, the ionic strength is a very important factor[19]. The effect of different ionic strengths on the extraction rate of anthocyanins is shown in Figure 3. For the composite system, when the potassium chloride concentration increased to 6 mmol/L, the extraction rate rose to 24.32%, but when the concentration continued to increase, the extraction rate of anthocyanin decreased again. The mechanism is that the chloride ion and the anthocyanin are linked to the head group of the SDS, and the increase of the ionic strength will cause the competition between the chloride ion and the anthocyanin, which will reduce the number of complex systems and thus lead to the anthocyanin. As a result, the recovery of anthocyanins is reduced.

3.4. The effect of potassium chloride dosage on the extraction rate of anthocyanin in apple peel

The increase of a certain amount of potassium chloride will promote the formation of a complex system, and the excessive amount will not be conducive to the formation of a complex system[20]. Therefore, the choice of the amount of potassium chloride is also one of the important variables of this
technology. Several groups of variables selected in this article are relatively small amounts of potassium chloride, so that unaffected the significant decrease of the critical micelle concentration of SDS caused by the addition of potassium chloride[21].

In the six groups of experiments, the amount of 6mmol/L KCl solution was 0.5mL, 1mL, 1.5mL, 2mL, 2.5mL, 3mL, respectively. Among them, the dosage of the same pH crude anthocyanin in each group was 3ml; The amount of acetone with a volume fraction of 5% was 3mL and the amount of NaCl solution for 0.25mol/L was 1mL.

![Fig. 4. Effect of Potassium chloride dosage on the extraction rate of anthocyanin in apple peel](image)

From Fig. 4, although the change of potassium chloride is trace, the effect of extraction rate of anthocyanin is very obvious. When the dosage of 6 mmol/L potassium chloride is 1.5 mL, the extraction rate of anthocyanin reaches 23.77%. The extraction rate of post-anthocyanin over 1.5 mL decreased instead. This may be the fact that the anthocyanin also absorbed some of the anthocyanins when the supernatant was drawn, resulting in a decrease in the extraction rate.

3.5.Effect of pH on the extraction of anthocyanin in apple peel
Since a strong alkaline environment is harmful to the effective binding of SDS and anthocyanins[22], it is considered to set the pH variable to be less than seven pH for experimentation.

The pH of the solution was controlled with hydrochloric acid and sodium hydroxide, setting six groups of anthocyanin crude solution pH 1,2,3,4,5,6 experiments.

Among them, the dosage of the crude anthocyanin in each group was 3ml; The amount of KCl solution for 6mmol/L was 1.5mL; The amount of acetone with a volume fraction of 5% was 3mL and the amount of NaCl solution for 0.25mol/L was 1mL.

![Fig. 5. Effect of pH on the extraction rate of anthocyanin in apple peel.](image)

SDS is an anionic surfactant and is a sulfonate. It does not hydrolyze in acidic media. As is shown in Figure. 6, when the pH is raised from 1 to 2, the extraction rate of anthocyanin soars from 29.57% to 49.42%, indicating that the strong acidic environment is not conducive to the extraction of anthocyanins from the system. When the pH is between 2 and 5, the system does not change significantly with the change of pH value, so it is sensitive to the environmental pH value. It isn’t high.
The reason may be that pH has little effect on sodium dodecyl sulphonate in this range, so the extraction rate of anthocyanins is not much different. When the pH rises to 6, the extraction rate of anthocyanin is reduced because SDS is in a weakly alkaline environment and its head group becomes inflexible[23], resulting in a decrease in the extraction rate of the final anthocyanin.

3.6. Effect of recovery solvents on the extraction of anthocyanin in apple peel
Setting three groups of recovery solvents were acetone, ethanol, methanol (all were analytically pure, and the amount is 3mL).

Among them, the dosage of the crude anthocyanin in each group was 3ml; The amount of KCl solution for 6mmol/L was 1.5mL and the amount of NaCl solution for 0.25mol/L was 1mL.

Table 6. Effect of recovered solvents on the extraction rate of anthocyanin in apple peel

| Recovery solvent | Acetone | Ethanol | Methanol |
|------------------|---------|---------|----------|
| Anthocyanin crude A | 0.786   |         |          |
| Extracted solution A | 0.477  | 0.146   | 0.143    |
| Extraction rate % | 60.69   | 18.58   | 18.19    |

In the demonstration of active agent precipitation technology, the choice of recovery solvent is very important, which directly affects the efficiency of this process[24]. In this study, three recovery solvents were selected. It can be seen from Table 6 that the crude anthocyanin solution with acetone as the recovery solvent has a significantly higher extraction rate of anthocyanin than ethanol and methanol as recovery solvents. For the SDS-anthocyanin complex system, when acetone is used as a recovery solvent, the extraction rate of anthocyanin can reach 60.69%. Therefore, acetone is the best recovery solvent. The following experiments use acetone as the recovery solvent.

3.7. Effect of acetone on extraction rate of anthocyanin in apple peel
Setting six groups of acetone with volume fractions of 20% for 1 mL, 1.5 mL, 2 mL, 2.5 mL, 3 mL, 3.5 mL.

Among them, the dosage of the same pH crude anthocyanin in each group was 3ml; The amount of KCl solution for 6mmol/L was 1.5mL and the amount of NaCl solution for 0.25mol/L was 1mL.

![Fig. 6. Effect of acetone dosage on the extraction rate of anthocyanin in apple peel.](image)

Although Fig. 6 shows that the extraction rate of anthocyanin is increasing along with the amount of acetone, the rate of increase is not significant. From the viewpoint of economy, the optimal amount of acetone should be 1 mL.

4. Conclusion
The effect of SDS surfactant precipitation on the extraction of anthocyanins from plants is significant and economically feasible. The results showed that the optimum experimental condition for extracting anthocyanin from the 3 mL apple juice crude solution was pH 2. The optimal reagent amount was 4...
mL 4 mmol/L SDS, 1.5 mL 6 mmol/L potassium chloride, and 1 mL volume fraction was 50%. Acetone, 1mL 0.25mol/L sodium chloride. This experimental scheme can make the extraction rate of anthocyanin reach 60.81%. China produces a large amount of peel waste every day. This technology has transformed these peel wastes into useful resources from another source. Experimental reagents are commonly used reagents in the laboratory, and it’s very convenient and economical. In general, this technology has the advantages of simple, effective, economical and so on.

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