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Short Communication

Simultaneous aqueous two-phase extraction and saponification reaction of chlorophyll from silkworm excrement

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ABSTRACT

Simultaneous aqueous two-phase extraction and saponification reaction of chlorophyll from silkworm excrement was investigated in this study, and it was compared with the saponification reaction followed by aqueous two-phase extraction (ATPE) method. The results indicate that it is better to use the simultaneous aqueous two-phase extraction and saponification reaction method than other method. The effects of different parameters on the amount of sodium chlorophyllin in the top system of the ethanol–NaOH aqueous two-phase system (ATPS) were studied, including salt concentration, saponification temperature and time. When the NaOH concentration was 0.4 g/ml, saponification temperature and time were 333.15 K and 2 h respectively, the amount of sodium chlorophyllin in the top system reached its maximum in the investigated range. Further, the top system of the ethanol–NaOH ATPS was taken out and used to prepare sodium copper chlorophyllin, and the absorbance ratio ($A_{405.00nm}/A_{627.00nm}$) was 3.67.

1. Introduction

As a kind of natural pigments, chlorophyll is abundant and widely available in the stem and leaves of green plants. The Nobel Prize Laureate Richard Willstatter and Hans-Fisher found that chlorophyll is structurally similar to heme. The chief difference is that the centre of the chlorophyll molecule is magnesium atom, while the centre of the heme molecule is iron atom. There are two main types of chlorophyll in higher plants, chlorophyll a and chlorophyll b. As shown in Fig. 1, the chlorophyll molecule is divided into two parts: a porphyrin ring and a long phytol chain [1]. The stability of the nature chlorophyll is so weak that it can be easily decomposed when encountering with heat, light, acid and alkali conditions, which limits its practical application. Sodium copper chlorophyllin (as shown in Fig. 2 [2]), a semi-synthetic derivative of chlorophyll, has the advantages of good stability and water-solubility over chlorophyll [2,3]. Sodium copper chlorophyllin is currently not only used extensively as food, cosmetic and pharmaceutical coloring, but also used as a new type of health food additives due to its high nourishment and health function [4]. So that, the method for the synthesis of sodium copper chlorophyllin has a vital significance.

The conventional preparation process of sodium copper chlorophyllin is illustrated in Fig. 3. The extraction process is followed by the saponification reaction process. This preparation process has the shortcomings of complexity, high organic solvent, time and energy consumption, as well as comparatively poor quality. Therefore, the improvement of production technology is necessary.

Aqueous two-phase system (ATPS) is usually consisted of either two incompatible polymers or a polymer and a salt in water above a certain critical concentration [5]. In comparison to the traditional separation and purification techniques, aqueous two-phase extraction (ATPE) is famous for its high moisture, low interfacial tension of phases, high capacity and yield, biocompatibility [6] and non-toxic. According to its composition, the main ATPS fall into three different forms: polymer-based ATPS, ionic liquid (IL)-based ATPS and hydrophilic alcohol-based ATPS. In comparison with the polymer-based ATPS and ionic liquid (IL)-based ATPS, the alcohol-based ATPS has the advantages of low viscosity and low price. So far, the ATPS has been applied in the separation and purification of proteins [7–9], metal ion [10,11], antibiotic [12], cell [13], and plant pigment such as anthocyanin [14]. However, the extraction of chlorophyll and sodium chlorophyllin using hydrophilic alcohol-based ATPS has not been reported.

Silkworm excrement especially the dried silkworm excrement is full of chlorophyll. As raw material, silkworm excrement not only has the virtue of low cost, abundant resources, but also can be obtained without time and season limitation. It is a good use of waste materials to extract chlorophyll from silkworm excrement.

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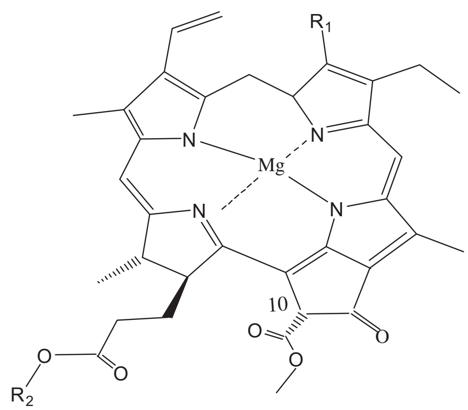

Fig. 1. Structure of chlorophyll. R_1 = CHO: chlorophyll (a) and R_1 = CH_3: chlorophyll (b); R_2 = phytyl.

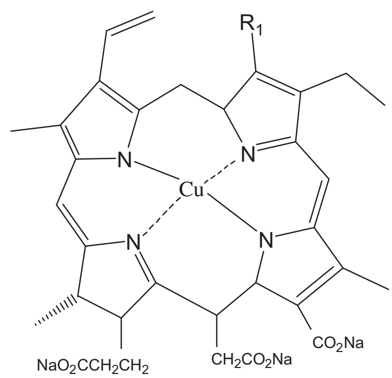

Fig. 2. Structure of sodium copper chlorophyllin. R_1 = CHO: chlorophyll (a) and R_1 = CH_3: chlorophyll (b).

The aim of this study was to develop a simple and convenient method for the preparation of sodium copper chlorophyllin, and the improved preparation process of sodium copper chlorophyllin is illustrated in Fig. 4. In this paper, a process of simultaneous ATPE and saponification reaction was applied to produce and extract the sodium chlorophyllin from silkworm excrement. Sodium chlorophyllin was synthesised by the saponification reaction between chlorophyll and sodium hydroxide in the ethanol–NaOH ATPS. This process does not only greatly simplify the operating steps, but also avoid the use of large quantities of organic solvent. To our knowledge, the current paper is the first in the integration of ATPE and saponification reaction. By discussing the influencing factors including the concentration of NaOH, saponification temperature and time, the optimal experimental conditions were obtained. Finally the top system containing sodium chlorophyllin was taken out and used to prepare the sodium copper chlorophyllin.

2. Materials and methods

2.1. Chemicals

Sodium hydroxide, ethanol, acetone, hydrochloric acerbic, petroleum ether and copper sulfate were supplied by Sinopharm Chemical Reagent Co., Ltd., with a minimum purity of 99.0%, 99.7%, 99.5%, 37.2%, 99.9%, 99.5%, respectively. All the chemicals used were of analytical grade, and double distilled deionized water was used throughout the entire experiment.

2.2. Preprocessing of silkworm excrement

The fresh silkworm excrement were dried in the sun and kept in the dry place. Before experiment, the silkworm excrement was ground and sieved by 120 meshes, then collected and subjected to the experiment.

2.3. Simultaneous ATPE and saponification reaction

An ATPS was prepared as follows: first, a prepared determined quantity of sodium hydroxide was dissolved in 50 ml water; second, a certain volume of NaOH solution mentioned above and ethanol were added to form the ethanol–NaOH ATPS, and the total volumes is 10 ml. Then the preprocessed silkworm excrement was added into the prepared ATPS. The mixture was shaken for 15 min, and placed in the water bath at constant temperature for a given time. The top system was taken out and diluted to 50 ml, and then determined by UV–Vis spectrophotometer at 639.00 nm to discuss preliminarily the optimal experiment factors including the concentration of NaOH solution, saponification temperature and time. In this process, the simultaneous ATPE and saponification reaction were carried out, and the hydrophobic chlorophyll was transformed into hydrophilic sodium chlorophyllin in the ethanol–NaOH ATPS.

2.4. Saponification reaction followed by ATPE

Saponification reaction followed by ATPE was also performed in this paper in order to compare with the simultaneous ATPE and saponification reaction method. It was performed as follows: the preprocessed silkworm excrement was added into the NaOH solution with different volume shaking for 15 min, and placed in the water bath at constant temperature for a given time, then ethanol with different volume was added respectively. The top system was taken out and diluted to 50 ml, and then determined by UV–Vis spectrophotometer at 639.00 nm.

2.5. Discussion of the optimum extraction conditions by the simultaneous aqueous two phase extraction and saponification reaction method

In order to obtain the optimum extraction conditions, the effect of NaOH concentration (0.36–0.7 g ml^{-1}) on the amount of sodium chlorophyllin in the top phase of ATPS was investigated at a fixed volume ratio of 2.33, and saponified for 2 h with different mass of silkworm excrement (0.1 g, 0.08 g and 0.05 g) at T = 333.15 K. The effects of saponification temperature (298.15–343.15 K) was investigated at a fixed volume ratio of 2.33, 0.40 g ml^{-1} NaOH concentration , and 2 h saponification time. The effects of saponification time (1–5 h) was investigated at a fixed volume ratio of 2.33, 0.40 g ml^{-1} NaOH concentration, and 333.15 K saponification temperature. All the experiments were carried out in triplicate.

2.6. Preparation of sodium copper chlorophyllin from saponated chlorophyll

On the basis of the discussed factors, the ATPS was magnified 100 times. The top system of the magnified ATPS was taken out and adjusted with 1:1 HCl to pH = 2–3, and 4 ml 10% $CuSO_4$ solution was added to the solution mentioned above, then the solution obtained was stir at 60–70 °C for 1 h. The precipitate was collected by suction filtration, and the crude copper chlorophyllin was obtained.

The crude copper chlorophyllin was removed of impurity by using an appropriate amount of distilled water, petroleum ether