AN OPTIMAL THERMAL CONDITION FOR MAXIMAL CHLOROPHYLL EXTRACTION

by

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This work describes an environmentally friendly process for chlorophyll extraction from bamboo leaves. Shaking water bath and ultrasound cleaner are adopted in this technology, and the influence of temperature of the water bath and ultrasonic cleaner is evaluated. Results indicated that there is an optimal condition for maximal yield of chlorophyll.

Key words: *bamboo leaves, shaking water bath, ultrasonic cleaner, chlorophyll extraction*

Introduction

Bamboo is an abundant, clean, and renewable resource on earth. The research of bamboo has gained increasing attention due to its worldwide availability and its immense potential use for various fields, including, food, construction material, and furniture. In recent years, we have witnessed a huge development of bamboo products appeared in the market. However, at the same time, we have to take a serious consideration about the waste byproducts from bamboo processing. The development of bamboo products could cause serious environmental problems if the byproducts are discharged into the environment randomly. Among them, residual bamboo leaves become the waste need to be concerned. Various application technologies have been developed in recent decades for improving the utilization of bamboo as well as its byproducts [1-3]. Our research work focuses on the utilization of bamboo leaves.

Chlorophyll is one of the main derivatives from the green plants and algae [4], which is found in abundance in bamboo leaves. It could be isolated from plant tissues through extraction. Chlorophyll absorbs light energy from the Sun and then transfers the energy to synthesize carbohydrates from CO_2 in photosynthesis processes [5]. Aside of its role in photosynthesis, chlorophyll shows applications in various areas. It is permitted as a natural coloring agent both in foods and cosmetics [6]. It also can be used as an internal deodorant and mouthwash due to its non-toxic property [4]. Furthermore, chlorophyll has long been considered to have health benefits to prevent chronic diseases such as cancer with regard to its anti-

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oxidant and anti-mutagenic properties. Meanwhile, it has also been used for therapeutic purposes, for instance, wound healing and anti-inflammatory applications [4]. The demand for chlorophyll has dramatically increased due to its wide applications.

In the present study, a new method with a combination of shaking water bath and ultrasonic cleaner was applied to extract chlorophyll from bamboo leaves with acetone solvent. An optimal thermal condition for maximal chlorophyll extraction was investigated.

Materials and methods

The extraction of chlorophyll was carried out in conical flask wrapped by aluminum foil to avoid light influence and make the reaction stable. Although chlorophyll is stable in nature, it could easily degrade due to heat, light, oxygen, acid, and enzymes when it is extracted from the plant tissue [7-9]. Bamboo leaves powders weighted 1 g were subjected to acetone solvent with a bath ratio of 1:40. The mixtures were extracted by shaking water bath and followed by ultrasonic cleaner. Finally the mixtures were filtered to obtain acetone extract. In order to understand the effect of temperature on chlorophyll extraction, different temperature settings of shaking water bath as well as ultrasonic cleaner were involved. Meanwhile, the influence of ultrasonic cleaner power towards chlorophyll yield was studied. Additionally, with the purpose to optimize the extraction processing, three main parameters, *viz*. the extraction temperature of shaking water bath, the temperature of ultrasonic cleaner, as well as the power of ultrasonic cleaner were selected for the three factor and three level orthogonal experiments.

Spectrophotometric measurements were performed by means of a T6 ultraviolet-visible spectrophotometer produced by Beijing Purkinje General Instrument CO., Ltd., Beijing. Extracted chlorophyll was diluted with 100% alcohol and then 1 ml diluted sample was transferred into cuvette to measure the absorbance value at 663 nm (A_{663}), which is directly proportional to the concentration of chlorophyll. Thus, A_{663} is used to evaluate the yield of chlorophyll under different extracting conditions. The silica gel G precoated thin-layer chromatography (TLC) plates (5 cm × 10 cm × 0.25 mm) from Branch of Qingdao Haiyang Chemical Co., Ltd., Qindgdao, China, were used for acetone extracts analysis. The mobile phase was composed of 3:1 (v/v) mixture of petroleum ether and acetone.

Results and discussion

Temperature is the key factor greatly influencing the extraction of chlorophyll from bamboo. In the present study, the extraction temperature of shaking water bath and ultrasonic cleaner were varied to evaluate its influence on the yield of chlorophyll.

For shaking water bath, the temperature was changed from 35 to 55 °C while maintaining other treating parameters the same. The change of absorbance value obtained from the spectrometer corresponded to the variation of the yield of chlorophyll. As can been seen in fig. 1, there was a rising trend in the absorbance value from 35 to 45 °C. And then, a downward trend of the absorbance value was observed from 45 to 55 °C. In other words, the yield of chlorophyll first increased and then decreased with the change of extraction temperature of shaking water bath. In the beginning, the low extraction temperature might lead to insufficient dissolving of chlorophyll. Therefore, less active chlorophyll molecules existed in the acetone solvent. The extraction efficiency was slow. With the increase of the extraction temperature, the ratio of dissolved chlorophyll in acetone was accelerated. However, excessively higher temperature would result in the decomposition of chlorophyll and finally reduce the active chlorophyll molecules in acetone. The highest yield of chlorophyll could be obtained at 45 °C.

For ultrasonic cleaner, the extraction temperature was varied from 30 to 55 °C while keeping other treating parameters the same. The relationship between the extraction temperature and chlorophyll yield was presented in fig. 2. Chlorophyll extraction improved with the increase in temperature up to 50 °C. The fastest increasing rate could be observed from 40 to 45 °C. A slight reduce in absorbance value could be found when the temperature reached 55 °C, indicating a lower chlorophyll yield appeared. In fact, the chlorophyll molecules were inactive when the extraction temperature of ultrasonic cleaner was too low, which led to insufficient dissolution of chlorophyll in acetone. In addition, the degradation of chlorophyll may become prominent at high temperature and prolong the extraction processing, which reduced chlorophyll extraction. Hence, the proper extraction temperature for ultrasonic cleaner was 50 °C. Furthermore, the influence of extraction power of ultrasonic cleaner was investigated, ranging from 80 to 180 W. It can be observed from fig. 3 that chlorophyll extraction improved with the increase in extraction power up to 140 W. Excessive energy may accelerate the decomposition of chlorophyll, and result in the lower extraction rate of chlorophyll.

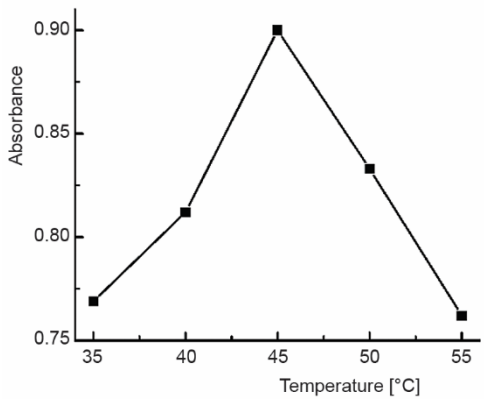

Figure 1. Effect of shaking water bath temperature on the yield of chlorophyll

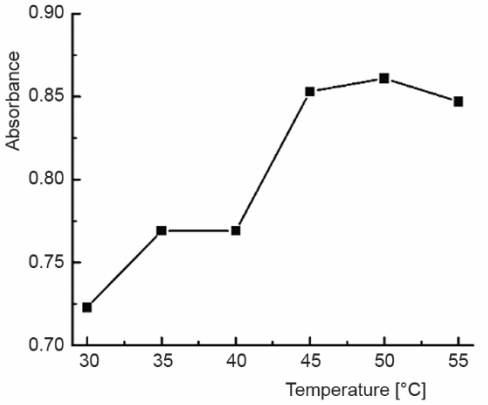

Figure 2. Effect of ultrasonic cleaner temperature on the yield of chlorophyll

For improving the chlorophyll extraction, an $L_9(3)^3$ orthogonal experiment was used to probe the interactions of the main parameters. In addition, the influence of extraction power of ultrasonic cleaner was investigated, ranging from 80 to 180 W. It can be observed from fig. 3 that chlorophyll extraction improved with the increase in extraction power up to 140 W. Excessive energy may accelerate the decomposition of chlorophyll and result in lower extract rate of chlorophyll. Our work pointed out that the factor of extraction temperature of shaking water bath (A) was a significant factor to effect chlorophyll extraction. The factor of extraction power of ultrasonic cleaner (C) was superior to extraction temperature of ultrasonic cleaner (B). Hence, the order of the effect on chlorophyll extraction was A > C > B. Besides, the optimal condition for maximal chlorophyll extraction was shaking water bath extraction temper-

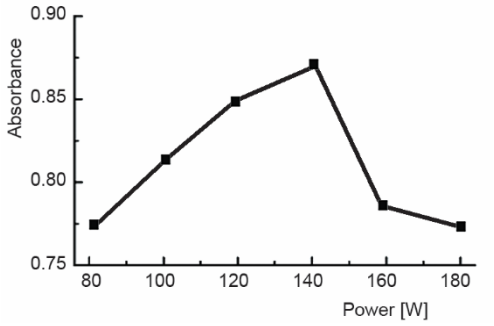

Figure 3. Effect of ultrasonic cleaner power on the yield of chlorphyll

ature of 50 °C, ultrasonic cleaner extraction temperature of 45 °C, and ultrasonic cleaner extraction power of 140 W. The result of TLC indicated that the acetone extract was formed by carotene, pheophytin, chlorophyll a, xanthophyll, chlorophyll b, and neoxanthin, among which, chlorophyll was the main constituent.

Conclusion

Bamboo chlorophyll can be successfully extracted from leaves by a new method with a combination of shaking water bath and ultrasonic cleaner. The optimized treating condition was: 50 °C for shaking water bath extraction and 45 °C for ultrasonic cleaner extraction with power of 140 W. Under this condition, the acetone extract yield reached highest. Temperature influenced the extraction efficiency significantly, since low temperature is not good for the active chlorophyll molecular dissolving in the acetone solvent while excessive higher temperature will cause chlorophyll degradation. It is concluded that the combination of shaking water bath and ultrasonic cleaner shows the possibility in chlorophyll extraction. Future investigations are needed on different extraction solvents to enhance the yield of the chlorophyll and the efficiency of extraction method.

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