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NOTE

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Rapid extraction of epidermis chlorophyll of moso bamboo (*Phyllostachys pubescens*) culm using ultrasonics

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Abstract The feasibility of applying ultrasonics to the chlorophyll extraction of bamboo culm was evaluated to establish an efficient reliable method for chlorophyll extraction from the epidermis of bamboo culm. The results demonstrate that extraction of epidermis chlorophyll from bamboo culm with acetone by ultrasonics for 3 min is a rapid, reliable method.

Key words Bamboo · *Phyllostachys pubescens* · Chlorophyll · Extraction · Ultrasonics

Introduction

The standing culm of bamboo has an attractive green color due to the chlorophyll in its epidermis. After drying, storage, or some other utilization processes, however, the chlorophyll on the bamboo culm surfaces is easily degraded and the green color fades, thereby reducing the economic value of the bamboo products. Fortunately, it has been possible to conserve the green color of bamboo culm by treatment with inorganic salts such as chromates, nickel salts, and copper salts.[1,2] Schanderl et al. had found that the bright-green complexes were formed during the storage of canned green vegetable purees at room temperature or above,[3,4] and it was established that the pigments were copper complexes mainly of pheophytin a and some pheophytin b.

Although there have been several investigations of this regreening phenomenon, sometimes from the commercial vegetable packing industry,[5] the mechanisms of color protection of bamboo culm have not been discussed to date. To elucidate the relation between inorganic salts and chlorophyll, it is imperative to establish an efficient, reliable method for chlorophyll extraction from the epidermis of bamboo culm. A variety of solvents, such as acetone, *N,N*-dimethylformamide (DMF), and dimethylsulfoxide (DMSO), have been used to extract chlorophyll from plant tissues in a number of studies.[6–10] Extraction of epidermis chlorophyll of bamboo culm, however, has not been mentioned in the literature.

Traditional methods of chlorophyll extraction involve grinding the plant tissues in solvents and subsequent centrifuging them to remove solid materials. It has been reported that these methods require a number of time-consuming procedural steps that inevitably result in experimental errors.[8,10] Wegener and Fengel demonstrated that milled wood lignin from spruce wood can be extracted within 14 h using ultrasonics, resulting in a yield and quality similar to that achieved with samples isolated by a 14-day shaking procedure.[11] The objective of this study is to evaluate the feasibility of applying ultrasonics to the extraction of chlorophyll from bamboo culm.

Materials and methods

The green epidermis of moso bamboo [*Phyllostachys pubescens* (Carr.) A. and C. Riv.] culm was ground to powder (particle diameter <0.7 mm) with a Wig-L-Bug grinder. Chlorophyll extraction of bamboo culm was carried out by the following methods. Green bamboo epidermis powder 40 mg was added to a sample vial containing 25 ml solvent (acetone, DMF, or DMSO); and chlorophyll was extracted either by grinding in a cold mortar for 3 min, using an ultrasonic washer for 3 min, or centrifugation for 30 min. After filtering the bamboo powder, the chlorophyll solutions were analyzed with an ultraviolet (UV)-Vis spectrophotometer.

Results and discussion

The traditional methods of chlorophyll extraction are time-consuming, and experimental errors are easily produced

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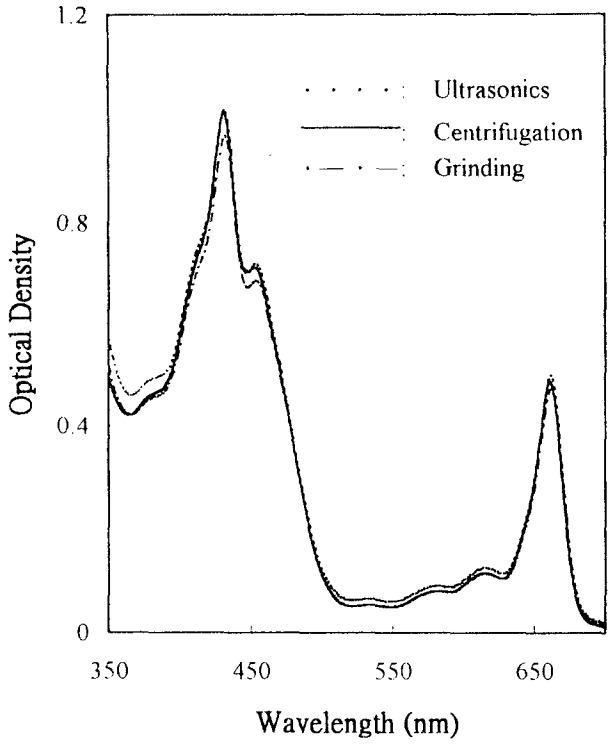

Fig. 1. Ultraviolet-visible spectra of chlorophyll of moso bamboo (*Phyllostachys pubescens*) culm obtained using different extraction methods

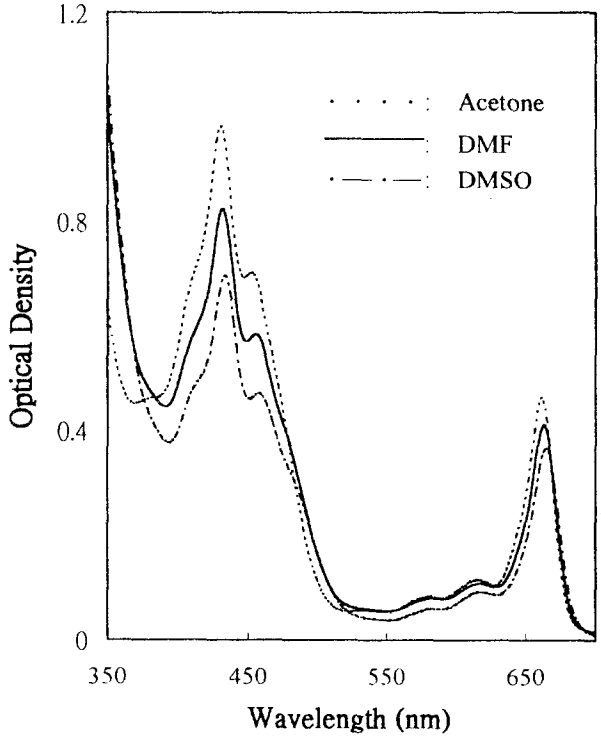

Fig. 2. Ultraviolet-visible spectra of chlorophyll of moso bamboo (*Phyllostachys pubescens*) culm extracted by ultrasonics in acetone, *N,N*-dimethylformamide (DMF), and dimethylsulfoxide (DMSO)

owing to artificial manipulation by grinding. In this study we tried to extract the bamboo chlorophyll by ultrasonics or centrifugation and to test the feasibility of avoiding the grinding method.

The UV-Vis absorbance spectra of chlorophyll extracted from bamboo powders in acetone by grinding, ultrasonics (3 min), and centrifugation (30 min) are shown in Fig. 1. The characteristic absorption peaks for chlorophyll a (662 and 431 nm) and chlorophyll b (642 and 452 nm) are readily recognized in the derivative spectrum of the chlorophyll extracts in acetone. Comparing the optical density (OD) of absorption peaks at 662 and 431 nm in Fig. 1, the results of the centrifugation method (662 nm, 0.504; 431 nm, 1.027) are almost equal to those using the ultrasonic method (662 nm, 0.499; 431 nm, 1.019). Moreover, the extraction results of these two methods were better than those when using the grinding method (662 nm, 0.472; 431 nm, 0.970). In addition, it was found that because of the toughness of epidermis chip of bamboo culm it is difficult to obtain complete chlorophyll extraction using the grinding method. Therefore it is indeed feasible to replace the grinding method with the ultrasonic method to extract chlorophyll from bamboo culm. Although centrifugation is also a good method for extracting chlorophyll from bamboo culm, it takes longer to achieve complete extraction.

To investigate extraction effectiveness we selected three commonly used solvents (acetone, DMF, DMSO) to extract epidermis chlorophyll from bamboo culm by ultrasonics. Figure 2 shows the UV-Vis absorbance spectra of epidermis chlorophyll in the three solvents. In decreasing order, the OD at 662 and 452 nm in the spectra of three solvents is as follows: acetone (0.462 and 0.699) > DMF (0.411 and 0.583) > DMSO (0.366 and 0.470). It was obvious that the amount of epidermis chlorophyll extracted with acetone was higher than that using the other two solvents. The results demonstrate that extracting epidermis chlorophyll of bamboo culm by ultrasonics in acetone for 3 min is an efficient, reliable method.

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