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An application of the alkaline extraction – glucoamylase hydrolysis method to analyze starch and sugar contents of bamboo

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Abstract The alkaline extraction – glucoamylase hydrolysis (AG) method, which extracts sugars and starch by using sodium hydroxide and hydrolyzes starch by using glucoamylase and α-amylase, was compared with the established ethanol extraction – perchloric acid hydrolysis (EPA) method to analyze the sugar and starch contents of moso bamboo (*Phyllostachys pubescens* Mazel). The results suggested that the two methods had comparable abilities to extract free sugars from moso bamboo. However, the AG method analyzed starch in moso bamboo more accurately than the EPA method under the proper conditions. When we take into account the better time performance of the AG method versus the EPA method, we can conclude that the AG method is superior to EPA for analyzing the sugar and starch contents of moso bamboo.

Key words Bamboo · Sugar · Starch · Alkaline extraction – glucoamylase hydrolysis method · Ethanol extraction – perchloric acid hydrolysis method

Introduction

Bamboo grows widely in tropical, subtropical, and temperate regions that have wet seasons. It grows faster than any woody plant, and its uses have, since ancient times, ranged from basic tools to furniture and building materials.

However, in conditions conducive to biological degradation, bamboo should be used with caution because its weak resistance to decay and insect attack reduces its durability. The biologically perishable properties of bamboo have been assumed to be mainly caused by the high content of sugar and starch, which act as feed for fungi or insects. Some reports have indicated that the contents of the three free sugars, i.e., glucose, sucrose, and fructose, and that of starch in bamboo depend on the season in which it is cut, although the results vary widely among these reports.[1–3] One of the major reasons for such variation in sugar and starch content might be the difficulty of extracting them from the bamboo.

Many methods have been suggested to extract sugar and starch from plants.[4–10] Among them, the ethanol extraction – perchloric acid hydrolysis method (EPA method), which extracts sugars from plants by using 80% ethyl alcohol and solubilizing starch by using dilute perchloric acid, is commonly used for estimating the sugar and starch contents of bamboo.[1,9] However, this method is time consuming and is reported to be relatively unreliable.[10] Therefore, a simpler and faster method is highly desirable.

In this study, the performance of the alkaline extraction – glucoamylase hydrolysis method (AG method),[7,10] which extracts sugar and starch by using sodium hydroxide and hydrolyzes starch by using glucoamylase and α-amylase, was compared with that of the established EPA method for analyzing the sugar and starch contents of bamboo.

Materials and methods

Materials

Three- to 5-year-old moso bamboo (*Phyllostachys pubescens* Mazel) culms were obtained from a bamboo plantation forest in Yawata City, Kyoto Prefecture, in May, June, and July 2002. Three culms were harvested for each month. The culms were cut so that each segment contained an internode. After pretreatment with acetone to remove the wax layer covering the epidermis on the outer surfaces, the cut segments were oven-dried at 65°C for 48 h. Bamboo specimens from the internodes were ground into a powder by using a vibration mill with a cutting rod to give an approximately 100-mesh particle size. Three replications were prepared for each sample.

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EPA method

Extraction and analysis of sugars

In an Erlenmeyer flask, 500 mg of the powder was mixed with 50 ml of 80% ethanol, and the mixture was sonicated at 405 W for 20 min (Branson, 5510J-DTH). The mixture was then refluxed for 20 min and, after cooling, was centrifuged for 10 min at 3000 rpm. The supernatant was recovered, and ethanol was evaporated in vacuo.

The glucose content in the water solution was analyzed with a glucose oxidase reagent kit (Glucose B-Test, Wako) by ultraviolet/visible (UV/Vis) spectroscopy (U-2001, Hitachi) at 505 nm.

Extraction and analysis of starch

Four milliliters of perchloric acid (5 + 3) was added to the extraction residue of the sugars, and the mixture was then ground in a mortar for 15 min to extract the starch. Distilled water was then added, and the mixture was centrifuged at 3000 rpm for 10 min to obtain a supernatant. This process was conducted a total of three times. The combined supernatant was heated in boiling water for 2 h to hydrolyze the starch. After neutralization by sodium hydroxide, the glucose content in the supernatant was analyzed by the same method described above. The amount of starch in the bamboo was calculated by multiplying the glucose content by 0.900.

AG method

Extraction of sugars and starch

Figure 1 shows the protocol of the AG method. In a centrifuging tube, 500 mg of the bamboo powder was mixed with 10 ml 0.5 N sodium hydroxide, and the mixture was sonicated at 405 W for 30 min. After neutralization by 0.5 N acetic acid, the mixture was centrifuged for 10 min at 3000 rpm. The supernatant was recovered.

Free glucose analysis

The glucose in the supernatant was analyzed with a glucose oxidase reagent kit as described above. To remove the inhibition of alkaline extracts, 20–30 mg of activated charcoal (Nacalai Tesque) was added to the solution, which was then allowed to stand for 10–15 min.[4] After centrifugation at 3000 rpm for 10 min, the supernatant glucose content was analyzed with the glucose oxidase reagent kit as described above (A in Fig. 1).

Starch analysis

Before the samples were analyzed, hydrolyzing conditions were examined with different enzyme concentrations and reaction periods. One milliliter of the supernatant was mixed with 1 ml sodium acetate buffer (0.1 M, pH 4.8) containing glucoamylase from *Rhizopus* sp. (Toyobo) and α-amylase from *Bacillus* sp. (Nacalai Tesque). The concentrations of glucoamylase and α-amylase were 10, 20, 30, and 40 mg per 10 ml. The solution was shaken at 120 rpm for 1, 2, and 3 h at 40°C. These enzymes specifically hydrolyze starch.

To remove the inhibition of alkaline extracts, 20–30 mg of activated charcoal (Nacalai Tesque) was then added to the solution, which was allowed to stand for 10–15 min.[4] After centrifugation at 3000 rpm for 10 min, the supernatant glucose content was analyzed with the glucose oxidase reagent kit as described above (B in Fig. 1). The amount of starch in the bamboo was calculated as (B − A) × 0.9.

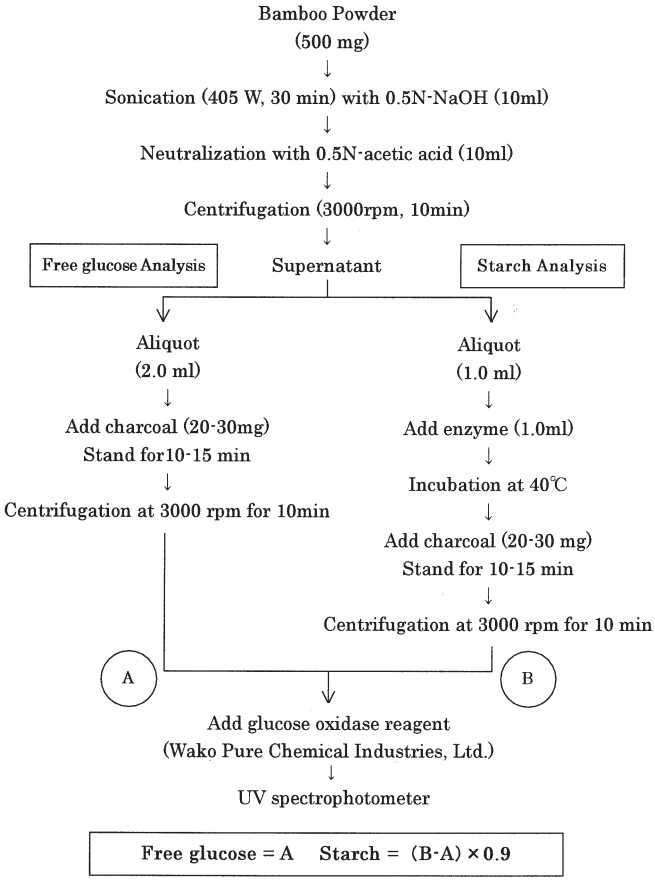

Fig. 1. The protocol of the alkaline extraction – glucoamylase hydrolyzation (AG) method

Results and discussion

Free sugar analysis

Sucrose, fructose, and glucose are the sugars contained in bamboo.[3] Previous studies showed that these sugars in bamboo were at their highest and lowest concentrations in spring and fall, respectively, and that their seasonal fluctuation was similar among the sugars.[3,11] Based on these

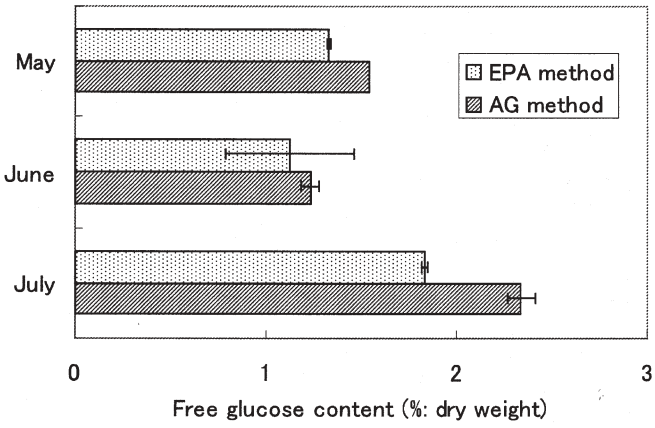

Fig. 2. Free glucose contents of moso bamboo extracted by the ethanol extraction – perchloric acid hydrolysis (*EPA*) method and *AG* methods. *Error bars* show standard deviations

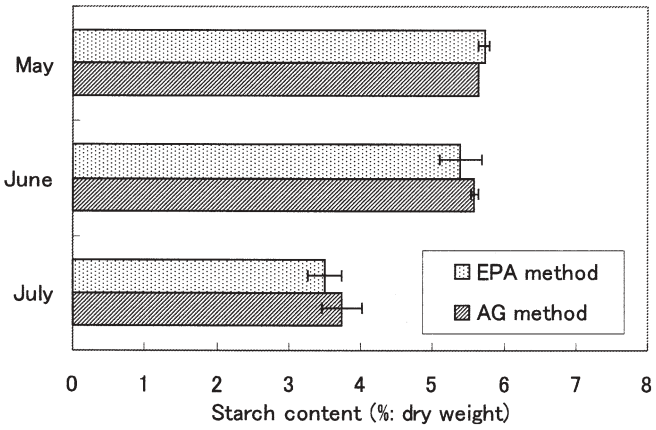

Fig. 4. Starch contents of moso bamboo analyzed by the *EPA* and *AG* methods

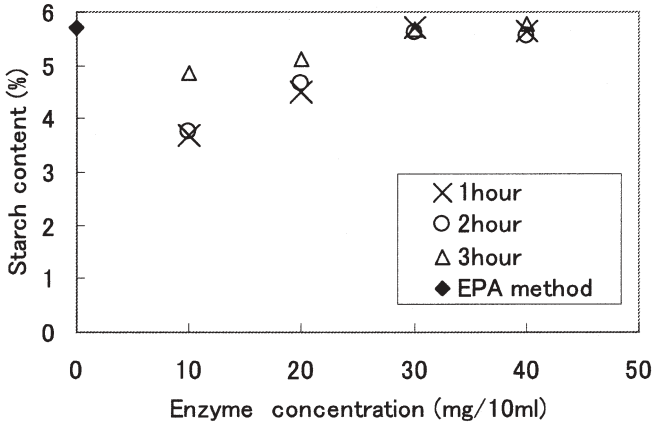

Fig. 3. The effects of enzyme concentration and reaction period on starch analysis by the AG method

findings, in this study we determined only the glucose content of bamboo. Figure 2 shows the free glucose content of moso bamboo extracted by the EPA method and that by the AG method. For the EPA method, the average free glucose contents of the specimens harvested in May, June, and July were 1.3%, 1.1%, and 1.8%, respectively. On the other hand, concentrations of 1.5%, 1.2%, and 2.3% free glucose were obtained with the AG method. Therefore, extraction using 80% ethanol might be insufficient to obtain sugars from moso bamboo, whereas alkaline extraction could be used for this purpose. Previous studies showed that glucose content in moso bamboo ranged from 0.57% to 3.51%, depending on the season.[3,12] The results of the present study are in the same range.

Starch analysis

The effects of enzyme concentration and reaction period on starch analysis in the AG method are shown in Fig. 3. With the lower enzyme concentrations, 10 mg/10 ml and 20 mg/10 ml, the starch contents were 3.6% – 5.1%, which were lower than the results obtained by the EPA method (5.7%), regardless of the reaction period. On the other hand, the higher enzyme concentrations, 30 mg/10 ml and 40 mg/10 ml, were strong enough to analyze starch even at the 1-h reaction period, showing starch contents of 5.6% – 5.7%, similar to that obtained by the EPA method. Therefore, the next starch analysis by the AG method used a 40 mg/10 ml enzyme concentration and a 2-h reaction period.

Figure 4 shows the starch contents of moso bamboo analyzed by the EPA and AG methods. With the EPA method, the starch contents of the specimens harvested in May, June, and July were 5.7%, 5.4%, and 3.5%, respectively. With the AG method, the contents were 5.6%, 5.6%, and 3.7%, respectively. These results clearly suggest that the AG method is applicable to the analysis of starch in moso bamboo if the proper hydrolyzing conditions are employed. It was reported that starch contents in moso bamboo ranged from 2.2% to 11.2%, depending on the season.[3] The results of the present study were in this range, as were the glucose contents. The starch content in ma bamboo (*Phyllostachys bambusoides* Sieb. et Zucc.) was shown to range from 0.3% to 4.0%, depending on the season.[1,11] This suggests that the starch content of bamboo is species dependent.

The AG method is faster than the EPA method in extracting and analyzing sugars and starch in moso bamboo. For example, starch can be extracted in the EPA method only by repeated grinding of the sample with perchloric acid (5 + 3) in a mortar for 15 min, a process that takes about 1 h per sample. The AG method, on the other hand, can analyze many samples at the same time. Taking into account the time efficiency of these two methods together with the present analytical results, we can conclude that the alkaline extraction – glucoamylase hydrolysis method is superior to the ethanol extraction – perchloric acid hydrolysis method for analyzing sugars and starch in moso bamboo.

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