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Lauroylation of sago residue at normal temperature and characteristics of plastic sheets prepared from lauroylated sago residue

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Abstract In order to thermoplasticize sago residue and decrease the amount of energy necessary for the reaction, lauroylation of sago residue was attempted at room temperature. Plastic sheets prepared from lauroylated sago residue were examined and characterized. Sago residue could be prepared at room temperature and the ester content of the lauroylated sago residue was sufficient for thermoplasticization. Plastic sheets could easily be prepared from lauroylated sago residue and were found to be soluble for several solvents. The weight of the plastic sheets was decreased immediately after placed or buried on and in soil when tested for the initial 20 days. A biodegradation test using *Tyromyces palustris* and *Trametes versicolor* showed that the plastic sheet obtained in this experiment was biodegradable. The degradability of the plastic sheets for some enzymes was evaluated by chemical oxygen demand (COD). It was suggested that enzymes may be responsible for and the hydrolysis of ester bond and the main chain of starch and cellulose in lauroylated sago residue.
Key words: biodegradability, energy for reaction, lauroylation, plastic sheets, sago residue

サゴヤシデンプン抽出残渣の常温でのラウロイル化とラウロイル化サゴ残渣から調製したプラスチックシートの性質

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要約　サゴヤシデンプン抽出残渣の熱可塑化と反応の低エネルギー化を目的とし，サゴヤシデンプン抽出残渣の常温でのラウロイル化を試みた．また，ラウロイル化サゴ残渣からプラスチックシートを調製し，その性質を調べた．本実験で用いた反応系において，常温でのラウロイル化反応は可能であり，プラスチック化に十分なエステル化度が得られた．プラスチックシートはラウロイル化サゴ残渣から容易に製造でき，各種溶媒に可溶であり，特にアルカリ（1N水酸化ナトリウム水溶液）への溶解性は著しく高かった．土壌環境中で分解を試みた結果，試験開始から20日間で著しく質量減少した．カワラタケおよびオオウズラタケによる分解性試験でも質量減少が生じ，生分解性であることが示された．リパーゼ，α－アミラーゼおよびセルラーゼによる酵素的分解を試み，その溶液の化学的酸素要求量（COD）で分解性を評価した．その結果すべての酵素でCODは増加し，ラウロイル化サゴ残渣はエステル結合の分解とセルロース，デンプンの主鎖の分解が起こりうることが明らかになった．上記の結果からエステル化反応のエネルギーの低減化とサゴ残渣からの生分解性プラスチックの製造が可能であることが明らかとなった．
キーワード　サゴヤシデンプン抽出残渣，低エネルギー反応，生分解性，プラスチックス，ラウロイル化

Introduction

It has recently become apparent that the development of new materials, which don't give the effect on the environment, is one of the methods to conserve the environment. For this purpose, a biodegradable plastic was prepared by chemical modification of sago residue, which is a natural resource and an agricultural waste product (Watanabe et al. 1997, Sasaki et al. 1999). However, to decrease the effect on the environment further and to realize the spirit of "green chemistry" (Anastas et al. 1996), the reaction factors such as temperature, catalyst and time in addition to the raw material must also be taken into account in a chemical manufacturing process of the material. The chemical modification of a natural resource such as wood or sago consumes considerable energy in conventional methods (Shiraishi et al. 1986, Watanabe et al. 1997, Sasaki et al. 1999). Therefore, chemical processing of sago residue at normal temperature was attempted in order to decrease the energy consumption of the reaction. In this study, sago residue was esterified with lauroyl chloride at room temperature and the biodegradability of plastic sheet prepared from the reaction product was examined. The reactivity at normal temperature and the characteristics of plastic sheets prepared from the reaction products are discussed.

Materials and Methods

Materials

Dry sago residue was supplied by Nitei Sago Industry (Sarawak, Malaysia). Sago residue was extracted for 24 hours with an ethanol-benzene (1:2 V/V) mixture and dried at 60℃ for 24 hours *in vacuo* before the reaction.

Lauroylation of sago residue

Oven-dried sago residue (10g) was soaked and swollen in a mixture of N, N-dimethylformamide (DMF, 150ml) and pyridines (150ml) for 1 hour at normal temperature. Thereafter, lauroyl chloride (300ml) was added and the sago residue was stirred and reacted for 24 hours at room temperature. After the reaction, the sago residue was rinsed 3 times with 67% ethanol aqueous solution and filtered. To remove the unreacted agent, sago residue was extracted with ethanol for 24 hours (Funakoshi et al. 1979). The ester content and chemical structure of the lauroylated sago residue were analyzed by the saponification method and by infrared spectrometry, respectively.

Preparation of plastic sheets

Plastic sheets were prepared by hot pressing the lauroylated sago residue in a steel-molding box. Hot-pressing conditions were the following: press pressure: 215kPa; press temperature: 160℃; press time: 1 hour. The pressure was maintained until the temperature had returned to room temperature after hot pressing. The target thickness and density of the plastic sheets were 0.3mm and 1.0 g/cm^3, respectively.

Measurement of modulus of elasticity

The modulus of elasticity (MOE) of the plastic sheets was measured by nonresonant, forced, and fixed frequency oscillation at 50 Hz by viscoelastic spectrometer (Reology Co., Ltd., Kyoto, Japan).

Solubility for solvents

Dried and weighed powder (80-mesh sieve pass, ca. 0.3g), prepared from the plastic sheet was put into an L-shaped tube with 50ml water, 1N hydrochloric acid solution, 1N sodium hydride solution, DMF, ethanol, hexane, or benzene and was shaken in a 25℃-incubator for 24 hours. Weight decrease was calculated from the dry weights of the powder before and after the experiment.

Degradability of plastic sheets

Degradability in different soil environments

The measurements of the degradability of the plastic sheets in different soil environments were carried out in the nursery of Tokyo-University of Agriculture and Technology (Fuchu, Tokyo, Japan). Samples (0.4 × 2.5 × 0.1cm) were packed in 5-mesh

and 24-mesh polyethylene bags and were placed on the soil surface and samples packed in 24-mesh bags were also buried in the soil at a depth of 5cm. The dry weight of the samples was measured every 20 days until the end of the experiment at 60 days.

Biodegradability by fungus (JIS 2000)

Two kinds of fungi, *Tyromyces palustris* (FFPRI 0507) and *Trametes versicolor* (FFPRI 1030), were cultured on potato dextrose agar medium for 20 days at 28℃. Thereafter, the samples (2 × 2 cm), which were sterilized and packed in 5-mesh polyethylene bags, were placed on each fungus mat and incubated for 60 days at 28℃. Dry weight decrease was calculated from weights before and after the experiment.

Degradability by enzymes

To examine degradability by enzymes, powder samples were prepared by milled the plastic sheet into an 80-mesh sieve pass and then sterilized for 48 hours at 60℃. The enzymes used in this study were four kinds of lipases, α-amylase, and cellulase; the enzymes and their origins and activities are shown in Table 1. Samples (0.1 g) and enzymes (100 μg) were placed in L-shaped tubes with 2ml sterilized phosphoric acid buffer (KH2PO4/Na2HPO4, pH=7.0) for the lipases and α-amylase, and 2ml sterilized acetic acid buffer (CH3COOH/CH3COONa, pH=5.0) for the cellulase. Samples were shaken in a 37℃-incubator for 3 days. The degradability of the samples by applied enzymes was evaluated by chemical oxygen demand (COD) of the filtrates (JIS 1981).

Results and Discussion

Characteristics of lauroylated sago residue

The color of untreated sago residue was light brown, while lauroylated sago residue was cream colored after the reaction; additionally, lauroylated sago residue was bulkier than untreated residue.

Figure 1 shows the IR spectra of both unreacted and lauroylated sago residue. The adsorption peak related to the stretching vibration of the hydroxyl group at 3620 cm^{-1} was decreased in the lauroylated residue while adsorption peaks related to the stretching vibration of the ketone group in ester bond at 1735 cm^{-1} and of the methylene group at 2925 cm^{-1} were increased. These results clearly show that ester bond was formed by the reaction of sago residue and lauroyl chloride with alkyl chain.

The weight percent gain of lauroylated sago residue was 47.9 % (standard deviation (SD): 5.6) and the ester content calculated from the results of the saponification method was 25.2 % (SD: 2.9). These values were smaller than those of sugi (*Cryptomeria japonica* D. Don), whose main component is cellulose (Funakoshi et al. 1979). Sago residue still contains not a little amounts of starch even after starch is extracted (Watanabe et al. 1997). Cellulose is a liner polymer composed of β-D-glucose, and starch is a mixture of amylose, which is spiral polymer of α-D-glucose, and amylopectin, which is a randomly branched

Table 1 Enzymes used in this study

Enzyme	Origin	Activity (units/mg)	Abbreviation
Lipase	*Rhizopus delmer*	730	RDL
	Candida cylindracea	30000	CCL
	Rhizopus arrhizus	50000	RAL
	Porcine pancreas	56000	PPL
α-amylase	*Aspergillus oryzae*	51	AOA
Cellulase	*Trichoderma viride*	10	TVC

polymer of α-D-glucose. Therefore, the different reactivity between sago residue and sugi might be due to differences in their components and stereoregularities. The reactivity of lauroyl chloride is higher than that of acetic anhydride (Watanabe et al. 1997) and palm oil (Sasaki et al. 1999), nevertheless it was possible for the reaction to take place at room temperature.

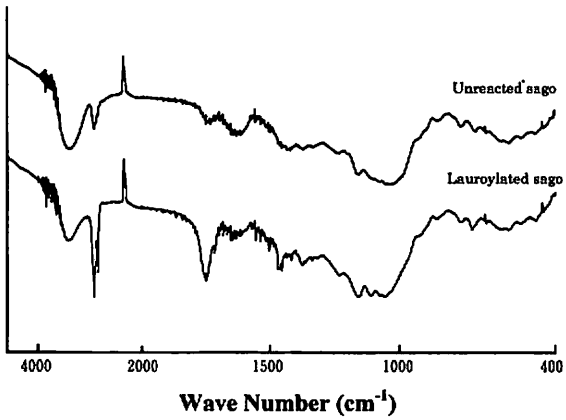

Fig. 1 Infrared spectra of unreacted and lauroylated sago residues

Preparation and properties of plastic sheets

Plastic sheets were easily prepared following the above hot press method without plasticizers. Plastic sheets prepared from lauroylated sago residue were clear and brown in color. The average density in oven-dry of the sheets was 1.0 g/cm³ (SD: 0.1). The average MOE of the plastic sheets was 1.2 GPa and plastic sheets showed higher elasticity than polyethylene (0.5 GPa); however, plastic sheets prepared from lauroylated sago residue were brittle.

Figure 2 shows the solubility of the plastic sheets for solvents. Plastic powder was soluble in all solvents used in this study, and weight decreases were 15-50 %. The solubility for 1N sodium hydroxide aq. solution was significantly higher than that of other solvents. 1N sodium hydroxide aq. solution, which is a solvent for starch, could be soluble with not only unreacted starch but also lauroylated starch, and a part of ester bond in lauroylated sago residue might be hydrolyzed. On the other hand, lauroylated sago residue was soluble for both polar solvents such as water, ethanol and DMF, and non-polar solvents such as benzene and hexane. The chemical components of sago residue are cellulose, hemicellulose, lignin, and starch, all of which are, in principle, insoluble with non-polar organic solvents. Lauroyl chloride reacted with hydroxyl groups of polysaccharides such as cellulose, hemicellulose, and starch as was shown in the results of IR spectrometry. Therefore, it was predicted that the lauroylated sago residue would be soluble with non-polar solvents and the solubilities for organic solvents were found to be almost equal because its ester content is not particularly high and the residue was partially lauroylated.

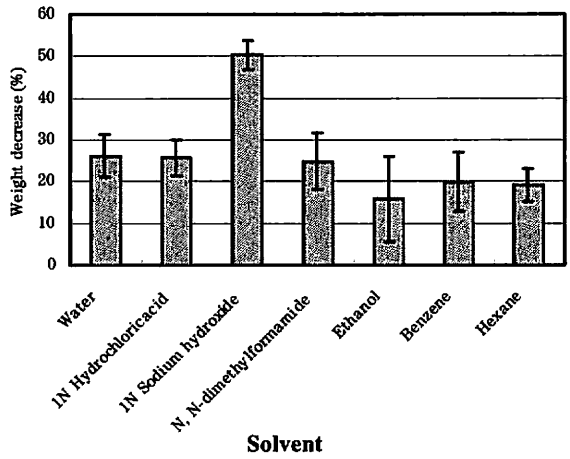

Fig. 2 Solubility of plastic sheets for solvents
Note: Vertical bars show standard deviations.

Degradability of plastic sheets

Degradability in a soil environment

Figure 3 shows weight decreases of plastic sheets with time when they were placed on soil surface. The weight of the plastic sheet decreased remarkably in the initial 20 days and continued to decrease slightly thereafter. The process of weight decrease of the plastic sheet was similar to that of a plastic sheet prepared from sago residue esterified with palm oil in a study by Sasaki et al. (2002). However, the decreases in weight of plastic sheets prepared with palm oil and lauroylation were 25-42 % (Sasaki et al. 2002) and 17-19 %, respectively, and the degradability of the plastic sheets prepared from lauroylated sago residue was smaller. No significant difference in

degradability was found to be related to the mesh size of the polyethylene bag.

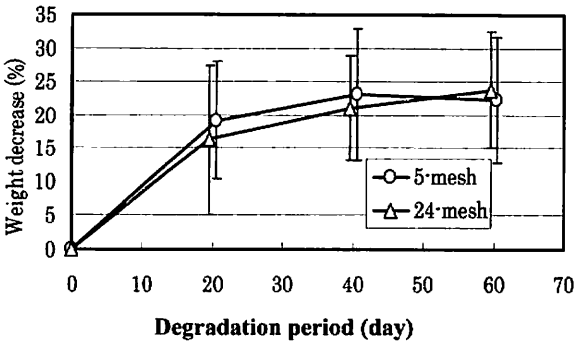

Fig. 3 Weight decrease of plastic sheets on soil
Note: Vertical bars show standard deviations

Figure 4 shows the weight decrease of the sample buried in soil. The weight of the sample decreased in the initial 20 days, showing a similar tendency to that of samples on the soil surface. Degradability was found to be higher in soil, with a weight decrease of 22 % at 20 days while the samples on the soil surface showed a decrease of only 17-19 % in the same time period. It is suggested that the degradability of plastic sheets depends on ester content and ester group because the weight decrease of the plastic sheet prepared from sago residue esterifed with palm oil was 55 %.

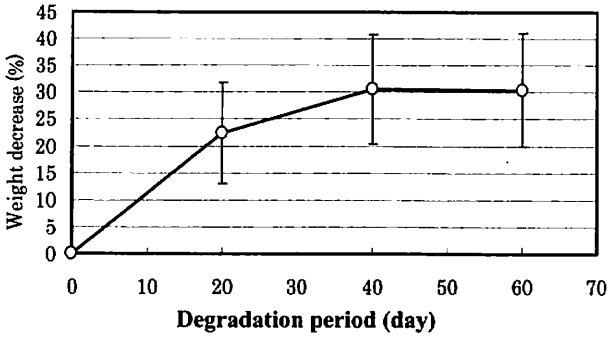

Fig. 4 Weight decrease of plastic sheets in soil
Note: Vertical bars show standard deviations

Biodegradability by fungus

Figure 5 shows the biodegradability of plastic sheets by *T. palustris* and *T. versicolor*. It is obvious that these microorganisms are able to biodegrade starch because the microorganisms were pre-cultured on the medium containing potato powder. The weight decreases of test samples were larger than those of control samples without microorganisms, though according to a test for the solubility of the sample for solvents, a part of the plastic sheet was soluble in water containing in the medium. Thus, the plastic sheet prepared from lauroylated sago residue was biodegradative. The activity of *T. versicolor* for the plastic sheet is slightly higher than that of *T. palustris*. *T. palustris*, which is known as a brown rot fungus for wood, primarily degrades crystalline region of cellulose with hydroxyl radical (Hirono et al. 1997), and then, enzymatically degrades cellulose. On the other hand, it is well known that *T. versicolor*, which is a white rot fungus, can enzymatically degrade lignin before cellulose and hemicellulose. Glucuronoxylan, a kind of hardwood hemicellulose, contains ester bond such as the acetyl group in its chemical structure. *T. versicolor* can degrade not only cellulose, hemicellulose and lignin but also ester bond. The plastic sheet was biodegraded in our experiment, nevertheless, the mechanisms of biodegradation of these microorganisms for plant components are different.

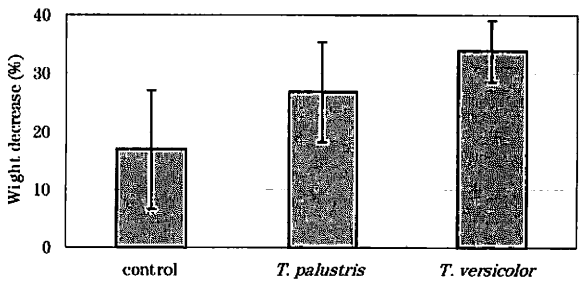

Fig. 5 Biodegradability of plastic sheets by fungi
Note: Vertical bars show standard deviations.
Control shows the result without microrganizms.

It has been shown that the biodegradability of chemically modified plant components depends on the degree of substitution (Charles et al. 1993). Sasaki et al. (2002) found a relatively high biodegradability for a plastic sheet prepared from esterified sago residue with palm oil, which showed a weight decrease of 75 % for *T. versicolor*. The biodegradability of the plastic sheets prepared in the

present study was lower because of the higher ester content.

Degradability by enzymes

Figure 6 shows the CODs (chemical oxygen demand) of sample solutions treated with enzymes. The CODs increased with enzyme treatments, and the plastic sheet was degraded with the enzymes. There are differences in degradability for some lipases. Lipase originating from *Rhizopus delmar* (RDL) shows the highest degradability, although its activity is lower than that of other lipases. On the other hand, porcine pancreas lipase (PPL) shows the lowest degradability in spite of its higher activity. The CODs of samples degraded by AOA and TVC increased. It was estimated that a certain percentage of the starch and cellulose in lauroylated sago residue was not esterified because of the rather low ester content. The degradability of hydroxyethylcellulose and carboxymethylcellulose by cellulase depends on the degree of substitution; specifically, it is easily degraded at two or more continuous unsubstituted glucose units in cellulose (Wirick 1968a, 1968b). Degree of substitution of glucose unit calculated from the weight percent gain and the result of component analysis (Sasaki et al. 2002) was 0.70 on the assumption that all lauroyl groups bonded to starch and cellulose, which is composed of glucose. Therefore, the degradation reaction with AOA and TVC might take place in unreacted starch and cellulose. Based on the above results, it was concluded that plastic sheets prepared from lauroylated sago residue could be degraded in both ester bond of the lauroyl group and by hydrolysis of starch and cellulose.

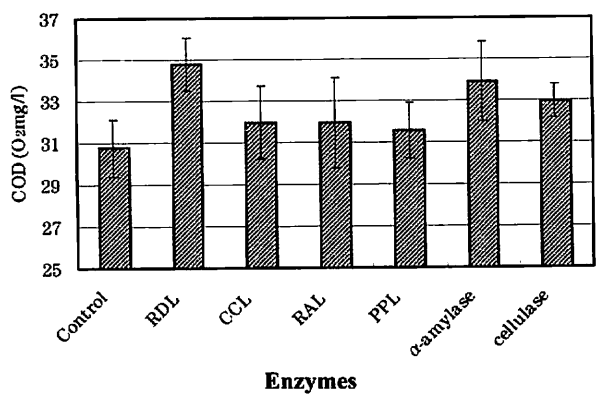

Fig. 6 Result of degradation test by enzyms

Conclusion

In order to thermoplasticize sago residue under the low energy consumption necessary for reaction, sago residue was estrified with lauroyl chloride at normal temperature. The reactivity at normal temperature and the characteristics of plastic sheets prepared from lauroylated sago residue were investigated and the following conclusions were obtained:

1) The weight percent gain of lauroylated sago residue was 47.9% and it was found that the reactivity of lauroyl chloride was found to be higher than that of acetic anhydride and palm oil; nevertheless, it was possible to produce the reaction at room temperature.
2) A plastic sheet was easily prepared by hot pressing the lauroylated sago residue. The MOE of the sheet was 1.2 GPa.
3) The plastic sheet was soluble in water, 1N hydrochloric acid solution, 1N sodium hydride solution, DMF, ethanol, hexane and benzene. The solubility for 1N sodium hydride solution was significantly high.
4) The degradability of the plastic sheet prepared from lauroylated sago residue was evaluated. The weights of the samples were decreased significantly after the initial 20 days of the test period both on surface of and in soil. The degradability of the plastic sheet in soil was higher than that on soil.
5) Both *T. palustris* and *T. versicolor* were able to biodegrade the plastic sheet, and the degradation activity of *T. versicolor* for plastic sheet was higher than that of *T. palustris.*
6) The degradability of plastic sheets by lipases, α -amylase, and cellulase were evaluated by COD. The CODs of samples treated with all enzymes increased and it is suggested that these

plastic sheets can be degraded in both ester bond and main chain of amylose and cellulose.

The above results clearly show that it is possible to decrease the amount of energy necessary for the reaction and preparation of biodegradative plastics from lauroylated sago.

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