Synthesis, Characterization, and Biodegradability of Fatty-Acid Esters of Amylose and Starch

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ABSTRACT: A series of starch and amylose esters with different degrees of substitution and side-chain length were prepared and studied. The esters were prepared by acylation of the polysaccharide with the appropriate acid chlorides, such as octanoic, dodecanoic, and octadecanoic. The degrees of substitution were 0.54, 1.8, and 2.7. After preparation, the resulting esters were characterized by elemental analysis, ^{1}H nuclear magnetic resonance (^{1}H-NMR), Fourier transform infrared (FTIR), differential scanning (DSC), thermogravimetric analysis (TGA), contact angle, and water uptake measurements. Their mechanical properties and, in particular, the tensile strength and elongation at break depend on the side-chain length and on the degree of substitution. The extent of their biodegradability, after exposure to activated sludge, was assessed by weight loss measurements and scanning electron microscopy (SEM). It was found that these new materials are biodegradable, and the biodegradation rate decreases with increasing degree of esterification. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 74: 1440–1451, 1999

Key words: starch; amylose; esterification; biodegradation

INTRODUCTION

In the last few years, studies concerning the total or partial substitution of synthetic plastics by biodegradable materials have been increasing steadily.[1] Despite the efforts to recycle used plastics, recycling is neither practical nor economical for certain applications, such as waste bags, agricultural mulch films, or food packaging. For this kind of applications, plastics are expected to degrade into safe by-products after their usage under normal composting conditions.

Extended studies have been undertaken to replace partially or totally the synthetic nonbiodegradable plastics. Replacement of petroleum-based plastics with materials from agroresources, especially starch, is attractive from the standpoint of providing biodegradation properties to the end product. This replacement will permit us to conserve our petrochemical resources and to find out new nonfood uses of starch. Indeed, starch is inexpensive (about 10 cents per pound), is totally biodegradable, and is available in large quantities from certain crops produced in abundance beyond available markets.[2]

Starch in its granule form is unsuitable for most uses in plastics industry, mainly due to processing difficulties during extrusion or injection molding. For this reason, a technology has been developed in which a mixture of native starch, plant fibers, food additives, and water is coextruded and injected in molds. After demolding

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and humidity equilibration, a stable and flexible material is obtained.[3] However, this composite can only be used for the production of tray-like forms, in which, high mechanical properties are not required.

Injection molding of starch can only take place in the presence of high amounts of water,[4] which acts as a plasticizer, allowing starch to melt under milder temperatures and shear stress conditions. After removing the excess amount of water, however, the material becomes brittle, having high tensile strength (about 30 MPa) but very low elongation at break (4%). Recent investigations have shown that it is possible to produce drug delivery containers from starch and gelatin, in the presence of water by injection machines.[5,6] If, instead of water, glycols are used as plasticizers, a thermoplastic material can also be produced. But even in this case, the mechanical properties of the materials produced are very poor (especially in tensile strength), depending on the kind of plasticizer used.[7] Glycerol is the most effective plasticizer but still cannot prevent the degradation of starch macromolecules during plasticization. It was found that the decomposition depends on the amount of glycerol as well as on the temperature used.[8] At high glycerol amounts (about 43 wt %), the depolymerization is diminishing, and it is very small at temperatures between 130–150°C.

Starch modification is another attractive way to produce thermoplastic materials and is well known since the early 1940s.[9,10] One negative point in all those efforts was the molecular weight reduction of starch during modification, due to the high susceptibility of starch to solvents and acid chlorides or anhydrides used for the acylation. Lately, there is a renewed interest for the preparation of modified starches with acetate,[11] hydroxypropyl,[12] alkyl siliconate[13] and fatty-acid ester (C_4–C_6)[14] groups. The main aim is to produce a fully biodegradable thermoplastic material, which will have the appropriate properties (especially mechanical), for replacing, whenever possible, the nonbiodegradable plastics used in the plastics industry.

The present work is part of a more extended work undertaken by our laboratories, towards studying the thermomechanical behavior and the biodegradability of systematically hydrophobized starch products as well as their blends with low-density polyethylene (LDPE). In the present study, we have prepared and characterized starch- and amylose-esters with higher acid chlorides (C_8, C_{12}, C_{18}), thus having a longer side chain. These modified esters are expected to exhibit an increased hydrophobic character due to the incorporated long hydrocarbon pendant groups, which would slow down their biodegradation rate.

EXPERIMENTAL

Materials

The potato native starch used in the acylation (esterification reaction) was provided by the INRA of Nantes, France. The potato starch contained 19% amylose and 81% amylopectin on a dry basis and had a moisture content of 14% by weight. The amount of proteins and lipids in this starch was insignificant. Corn amylose (70%), sodium azide, and 3,5-dinitrosalycilic acid were purchased from Sigma Chemical Co. Lipase type II (220 U/mg at pH 7.7) and α-amylase type VI-B (25 U/mg at pH 6.9), both from porcine pancreas were purchased from Sigma. Buffer of phosphates 0.05*M,* pH 7.2 were prepared using analytical-grade reagents. The octanoyl, dodecanoyl, and octadecanoyl (stearoyl) chlorides (Aldrich) were of reagent grade. Pyridine used as an esterification solvent and absolute ethanol used for precipitation of the esters, were of SDS anhydrous analytical grade.

Esterification

The esterification of starch or amylose was carried out by the modified method of Mullen and Pacsu.[9] This method was preferred to others reported in literature because it requires only a minimal amount of organic solvent in the synthesis reaction. In addition to its catalytic effect, the organic solvent (pyridine) used, minimizes starch deterioration during esterification because it neutralizes the HCl formed during the reaction. The general procedure of esterification was as follows: The native starch or amylose was dried overnight in an oven at 105°C to remove moisture (final moisture $< 2\%$). Afterwards, 20 g were placed in a double-necked flask, equipped with a mechanical stirrer and a condenser. Subsequently, 150 mL of pyridine and the appropriate amount of chlorides were added. By changing the amount of chloride, it was possible to prepare esters with different degrees of substitution always taking into account that some part of the chloride is

Table I Reaction Conditions for Starch Esterification

Starch	Alkyl Chain	Starch Quantity (mol)	Chloride Quantity (mol)	ds
Native	C_8	0.123	0.28	1.8
Native	C_8	0.123	0.56	2.7
Native	C_{12}	0.123	0.56	2.7
Native	C_{18}	0.123	0.28	1.8
Native	C_{18}	0.123	0.56	2.7
Amylose	C_8	0.123	0.14	0.54
Amylose	C_8	0.123	0.56	2.7
Amylose	C_{12}	0.123	0.56	2.7
Amylose	C_{18}	0.123	0.56	2.7

Reaction time: 3 h at 105°C.

consumed by reaction with the remaining traces of water in starch. The experimental conditions for the esterification reaction are given in Table I.

Upon completion of the reaction, the soluble esterified product was isolated by precipitation in 500 mL of absolute ethanol and was washed twice with 500 mL hot ethanol to eliminate any color impurities and by-products. After filtration, ethanol was removed by an air stream, and the starch ester was dried at 50°C overnight and weighed. The yield for all products was practically 100%.

The produced esters are hereafter referred to as follows: The octanoated, dodecanoated, and octadecanoated products are indicated by the prefixes OC, DOD, and OCD, respectively. Modified starch or amylose are indicated by ST or AM, respectively, which follow the above prefixes. The degree of substitution is indicated by a number at the end. As an example, DODST2.7 means dodecanoated starch with a degree of substitution 2.7.

Characterization

Elemental analysis of the samples was performed on a Carlo Erba elemental analyzer, model 1106. Prior to measurement, samples were dried at 100°C for 24 h.

The ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectra of the samples were collected on a Brucker 200 MHz Fourier transform (FT) spectrometer. All spectra of esters were taken in deuterated chloroform solution. For pure starch and amylose, the spectra were collected in deuterated dimethyl sulfoxide solution.

Contact angle measurements of esters were done in accordance with the method described in a previous publication.[15]

Fourier transform infrared (FTIR) spectra were acquired in a Perkin–Elmer (Model 1610) FTIR spectrometer. For each spectrum, four consecutive scans with a 4-cm^{-1} resolution were averaged. Samples were cast on KBr windows from a chloroform solution. Spectra for pure amylose and starch were collected using the KBr pellet method.

Differential scanning calorimetry (DSC) measurements of samples were performed in a Shimadzu DSC-50Q fast-quenching differential scanning calorimeter. Samples were placed in sealed aluminum pans, using a quantity of about 10 mg for each sample. The samples were initially heated with a heating rate of 20°C/min from −120 up to 200°C in a nitrogen atmosphere and quenched immediately to remove any previous thermal history. The samples were subsequently rescanned with a heating rate of 20°C/min at the same temperature rate. From these thermograms, the melting and glass transition temperatures were determined.

Thermogravimetric analysis (TGA) measurements were performed in a Shimadzu TGA-50 Thermogravimetric Analyzer. Each sample was heated with a heating rate of 20°C/min in a nitrogen atmosphere up to 700°C. Prior to thermal analysis, the samples were dried in a vacuum oven at 80°C for 24 h.

Water absorption was measured by using 3×8 cm film strips of 80 μm thickness according to the ASTM D570-81 method. The measurements were performed by soaking the samples in distilled water. At regular time intervals, each sample was removed from the water tank, dried by wiping with blotting paper, and then weighed in an analytical balance with a precision of 0.0001 g to determine water uptake. The samples were placed back in water after each measurement. The water absorption was calculated as the weight difference and is reported as percent increase of the initial weight.

Measurements of the mechanical properties, such as tensile strength and elongation at break, were performed on an Instron mechanical tester, Model 1122, according to the ASTM D638 method. Measurements were done using a 5 mm/min crosshead speed. Prior to measurements, the samples were conditioned at 50 ± 5% relative humidity for 48 hours by placing them in a closed chamber containing a saturated $Ca(NO_3)_2 \cdot 4H_2O$ solution in distilled water (ASTM E-104). Five measurements were conducted for each sample,

and the results were averaged to obtain a mean value.

Biodegradation in Activated Sludge

The polymer blends in a form of thin films 130 ± 3 μm thick were exposed to activated sludge in a waste water treatment facility of a food company for 8 weeks. The test permitted us to evaluate the rate of aerobic biodegradability by measuring the weight loss and mechanical properties of the studied samples after 2, 4, 6, and 8 weeks of exposure to activated sludge. The temperature of the sludge (25 ± 1°C) and the pH (about 7) were kept constant during the 8 weeks.

Measurements performed to follow the biodegradation of the samples were the following.

- Sample weight was used to determine the starch weight loss. In fact, all samples showed a weight increase due to water absorption after immersing in activated sludge. We defined weight loss as the difference between the water absorption in pure water and in activated sludge at the same exposure time. In the second case, due to the starch consumption by the microorganisms, the % water absorption is always lower than in pure water.
- Scanning electron microscopy (SEM) was used, using a JEOL microscope, model JMS-840.
- Mechanical measurements, such as tensile strength and elongation at break, were performed as described above.

Enzymatic Hydrolysis

Octanoated starch (0.5 g) with different degrees of substitution, such as 0.54, 1.8, and 2.7, was ground and allowed to stay for 48 h at 58% relative humidity. A sodium azide solution (2%) was added to every enzymatic solution to avoid microbial growth prior to starting the measurements. The testing material was immersed into 25 mL of an α-amylase solution (40 mg/mL) and maintained at 30°C with magnetic stirring for 72 h. Then 25 mL of a lipase solution (of concentration 4 or 40 mg/mL) was added and maintained under the same operational conditions. A sample of 1.5 mL was taken each day for 4 days and deactivated by heating at 100°C for 15 min in order to measure the released glucose.

The reducing value (glucose) measurements were accomplished by a spectrophotometric assay using 3,5-dinitrosalycilic acid as a reagent. The biodegradation rate is expressed as the percent of glucose liberated versus time in relation with the total concentration of glucose into the octanoated starch.[16]

RESULTS AND DISCUSSION

The appearance of the produced esters, after their purification, depends on their degree of substitution. Those with high degree of substitution (ds) have the form of a fluffy yellowish mass and behave like thermoplastic materials, whereas those with a low ds have the appearance of a white powder. The degree of substitution for a starch or amylose derivative is defined as the moles of substituents of hydroxyl groups per D-Glucopyranosyl structural unit of the polymer. Since each repeating unit contains three hydroxyl groups, the theoretical maximum degree of substitution is three. Table I shows the calculated ds of the synthesized starch esters as determined by elemental analysis (Table II) and ^{1}H-NMR.

The chemical changes in the structure of starch and amylose were verified by ^{1}H-NMR and FTIR spectroscopy. The ^{1}H-NMR spectrum of the esterified starch shows the three protons of the terminal methyl group of the acyl chain as a triplet at 0.86 ppm (Fig. 1). The peaks between 1.23 to 1.67 ppm correspond to the 10 protons of the methylene groups in the acyl chain; whereas at a chemical shift of 2.2 ppm, it is observed the signal of the 2 protons of the α-methylene group. Additionally, the NMR spectrum of the esterified starch reveals the presence of the seven protons of the glycoside structure between 3.5 and 5.5 ppm that are found in the NMR spectrum of the native starch as well.

The FTIR spectra of native and esterified starch or amylose confirm the extend of esterification, as shown in Figure 2, depicting some representative spectra of amylose esters. In the spectrum of pure amylose, a strong broad band between 970–1200 cm^{-1} with three peaks is the most characteristic band for a polysaccharide and is attributed to C—O stretching. This band is also observed in amylose esters where the three peaks are better resolved. Another characteristic band is the one between 3000–3700 cm^{-1}, due to hydroxyl bond stretching. The intensity of this peak decreases in the esterified derivatives. This peak's maximum is shifted towards higher wave numbers, from 3391 cm^{-1} for pure amylose, to

Table II Properties of Synthesized Starch Esters

Starch Ester	Code Name	Elemental Analysis C%	H%	O%	Molecular Formula	Contact Angle (°)	Appearance
Octanoated starch, ds 1.8	OCST1.8	64.2	8.8	28.5	$C_{20.8}H_{34.2}O_{6.9}$	87	white powder
Octanoated starch, ds 2.7	OCST2.7	65.8	9.6	24.3	$C_{27.5}H_{48.2}O_{7.6}$	92	pale yellow mass
Dodecanoated starch, ds 2.7	DODST2.7	66.9	10.5	22.6	$C_{21.7}H_{51.4}O_{6.9}$	95	pale yellow mass
Octadecanoated starch, ds 1.8	OCDST1.8	70.9	9.9	17.5	$C_{38.6}H_{64.7}O_{7.2}$	93	pale yellow powder
Octadecanoated starch, ds 2.7	OCDST2.7	74.3	12	13.5	$C_{54.5}H_{105.7}O_{7.5}$	95	yellow powder
Octanoated amylose, ds 0.54	OCAM0.54	53.3	7.8	38.2	$C_{10.2}H_{17.9}O_{5.5}$	dissolves	white powder
Octanoated amylose, ds 2.7	OCAM2.7	65.8	9.6	24.3	$C_{27.5}H_{48.2}O_{7.6}$	83	pale yellow mass
Dodecanoated amylose, ds 2.7	DODAM2.7	66.9	10.5	22.6	$C_{21.7}H_{51.4}O_{6.9}$	90	pale yellow mass
Octadecanoated amylose, ds 1.8	OCDAM2.7	74.3	12	13.5	$C_{54.5}H_{105.7}O_{7.5}$	132	yellow powder

3459 cm^{-1}. This happens because there is a decrease in the concentration of hydrogen-bonded hydroxyls, as they are converted into ester groups during the reaction. An intense ester carbonyl band appears at 1746 cm^{-1} in the final products. The absence of a shoulder in lower wave numbers in this area verifies that hydrogen bonds between the remaining hydroxyl groups and the carbonyl groups of the esters are absent or very sparse. This band, as well as the band at 2800–2950 cm^{-1}, corresponding to methylene group deformation increase with the degree of substitution. The new peak that appears at 3022 cm^{-1} is due to the methyl groups of the ester.

The structural modification of starch or amylose has, as a result, a significant change of their physicochemical properties. As a consequence, the prepared esters show significant differences compared to unmodified polysaccharides used for their preparation. All produced esters are soluble in common solvents, such as chloroform, in contrast with starch, which is soluble only in warm DMSO. On the other hand, the most prominent feature of the esterified starches is their reduced hydrophilicity as determined by contact angle measurements (Table II). These values are comparable to that of poly(methyl methacrylate) (PMMA), which is a hydrophobic synthetic polymer and has a contact angle of 85°. The increased hydrophobicity of esterified esters is attributed to the replacement of hydrophilic hydroxyls by the relatively hydrophobic ester groups. Hydrophobicity is increasing with the degree of substitution, as can be seen in the case of octanoated and octadodecanoated starch. The contact angle is about 2–5° higher than the corresponding esters with lower degree of substitution. It increases

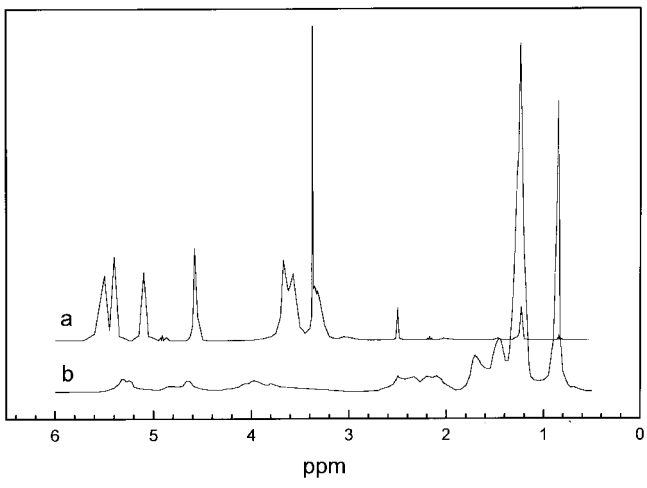


Figure 1 ^{1}H-NMR spectra of (a) pure starch and (b) octanoated starch with ds = 1.8.

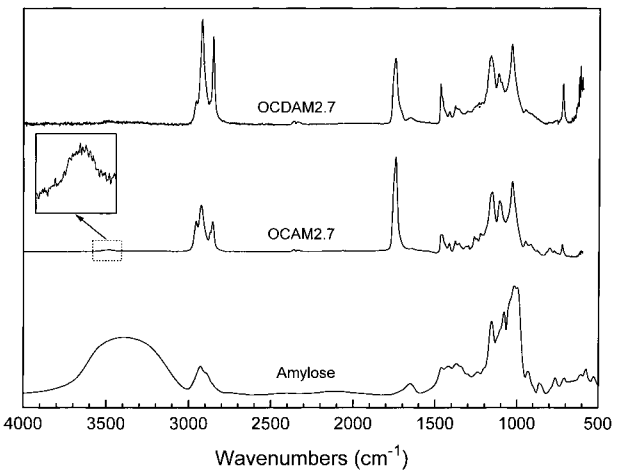


Figure 2 FTIR spectra of pure amylose and its octanoated (C_8) and octadecanoated (C_{18}) esters with ds 2.7.

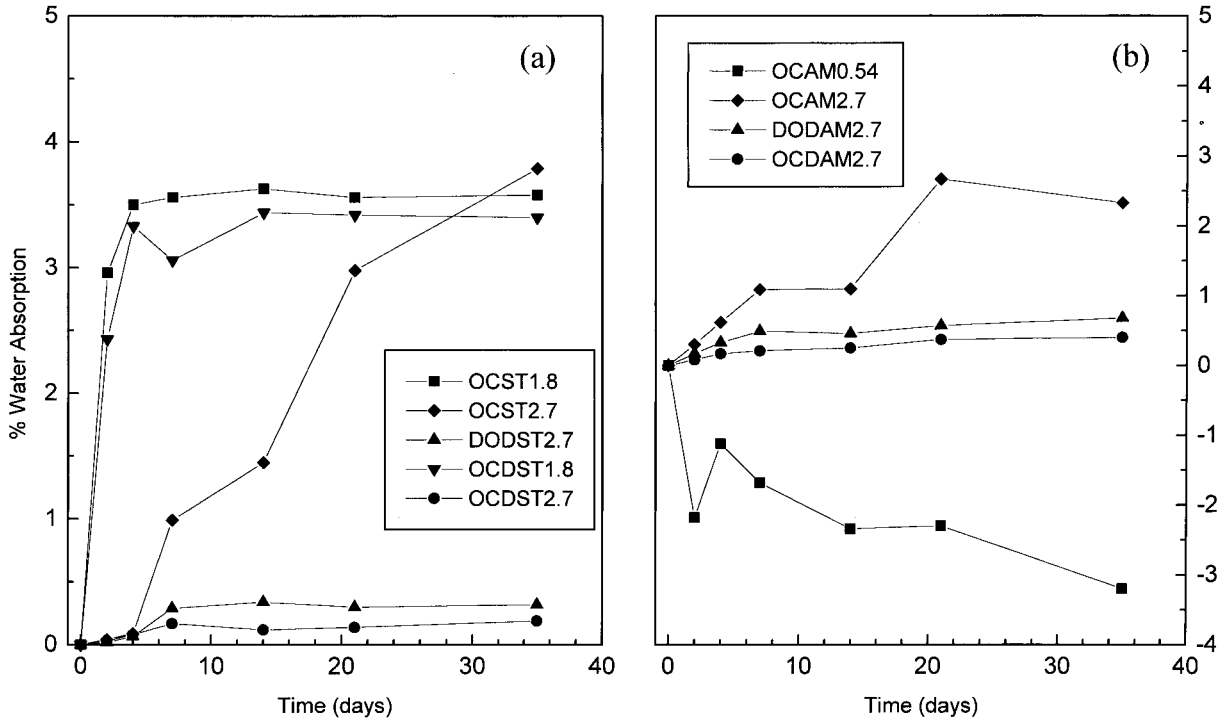


Figure 3 Water absorption versus time of (a) starch and (b) amylose esters.

also with side alkyl chain length. This is more clear in the case of amylose esters.

The loss of hydrophilic character is also reflected in water absorption measurements depicted in Figure 3.

The main factor affecting water uptake seems to be the degree of substitution. Thus, starch esters, like the octanoated and the octadecanoated with a degree of substitution 1.8 show significantly higher water absorption compared to the corresponding esters with a degree of substitution of 2.7. Even so, the maximum increase does not exceed 4 wt %, indicating essentially hydrophobic materials. Native starch has the ability to absorb water about four to five times its own weight, when it is immersed in water. It seems that even the replacement of about half of the hydroxyl groups in esters with ds 1.8 is enough for changing drastically the hydrophilic character of the starch. The water uptake occurs mainly within the first 5 days, and, afterwards, no significant water absorption takes place. It must be noted, however, that octanoated amylose with ds 0.54 shows a weight loss (about 3.3% of the initial weight) in the same period. Obviously, in such low degrees of substitution, the product still remains hydrophilic. As a result, it may swell and part of it can be partially extracted by cold water.

The side-chain length has also a minor effect upon the water uptake properties since water absorption seems to be decreasing with increasing side-chain length. Dodecanoated and octadecanoated esters with ds 2.7 show a similar behavior, which is different from that of octanoated esters. The later has a very small water absorption which seems to grow steadily, without reaching a plateau as in the case with esters having ds 1.8. This behavior could be attributed to the lower chain length of the ester, which cannot hinder effectively the unreacted hydroxyl groups; and, as a result, they can absorb water. Finally, amylose and native starch esters show similar water absorption characteristics. These results are in good agreement with those from contact angle measurements. The final conclusion is that the esters have low water absorption characteristics, making them appropriate for applications where water absorption must be minimal.

The produced starch and amylose esters are in their majority amorphous thermoplastic materials with a measurable glass transition temperature (T_g), especially those with a high degree of substitution. This is due to the loss of crystallinity of the starch and amylose after esterification. The T_g of granular starch, estimated from extrapola-

Table III Properties of Starch and Amylose Esters

Starch Ester	T_g (°C)	T_m (°C)	Tensile Strength (MPa)	Elongation at Break (%)
OCST1.8	68	174	3.1 ± 0.5	9 ± 1.6
OCST2.7	−50/40	—	0.7 ± 0.4	380 ± 33
DODST2.7	−56/25	—	0.7 ± 0.4	1500 ± 86
OCDST1.8	—	32	3.7 ± 0.6	9 ± 2
OCDST2.7	—	32	1.9 ± 0.3	10 ± 2
OCAM0.54	—	—	1.8 ± 0.4	9 ± 1.5
OCAM2.7	−52	—	1.2 ± 0.3	600 ± 50
DODAM2.7	−47	—	1.1 ± 0.3	1550 ± 45
OCDAM2.7	—	31	3.3 ± 0.8	19 ± 3

tion data, is about 210°C.[17] The DSC data of studied starch esters are presented in Table III.

In general, an increase of the side chain length causes a small depression of T_g (for the same degree of substitution). This trend is in accordance with the literature where the T_gs of lower starch esters are reported to drop from 65°C for starch butyrate to 50°C for starch hexanoate.[14] This behavior could be explained by the increase in the free volume of the polymer, which is caused by the introduction of bulky flexible side-chain groups. The loss of hydrogen bonding interactions, which often stiffen the macromolecular chains, also contribute to a reduction in T_g. The internal plastization due to the esterification is so effective that the T_g drops to very low temperatures (bellow −50°C). This is very close to the glass transition of thermoplastic starch (T_g = −38°C) containing 25 wt % glycerine as external plasticizer. It must be noted that some native starch esters seem to have two glass transition temperatures. These probably correspond to amylose and amylopectin esters, respectively, which, as their unmodified raw materials, remain incompatible after esterification. Since amylopectine is a branched molecule, it has a higher T_g than the linear amylose molecule,[18] and this must apply to their esters as well.

The loss of starch crystallinity after its esterification, as mentioned above, can be seen more clearly in the octanoated starch with degree of substitution 1.8 (OCST1.8). It shows a weak and broad melting peak at 174°C, a sign of imperfect crystallization. Unmodified potato starch is estimated to have a degree of crystallinity between 20–28% and melting peak between 220–230°C. The same behavior also appears in the octadecanoated (C_{18}) esters. In none of these esters, a glass transition was recorded, but all show a large melting peak around 32°C. Since this peak does not depend on the degree of substitution and the polymer type, it must be attributed to crystallization of the long C_{18} side chains. In fact, this melting peak is very close to the melting point of octadecane (28–30°C) and methyl stearate (40–42°C).

The thermal stability of the prepared esters was studied by TGA. From a previous study of ours in octanoated and dodecanoated starch esters, it was found that they have higher thermal stability than unmodified starch.[15] Amylose esters behave similarly (Fig. 4).

Pure amylose is stable up to 290°C. The maximum decomposition rate appears at 330°C. The amylose esters appear to be more stable since their decomposition starts at higher temperatures. Comparing the thermal stability of starch and amylose esters, no significant differences appear between them. This greater thermal stability of the esters is probably due to the lower amount of remaining hydroxyl groups after esterification. It has been reported that the main de-

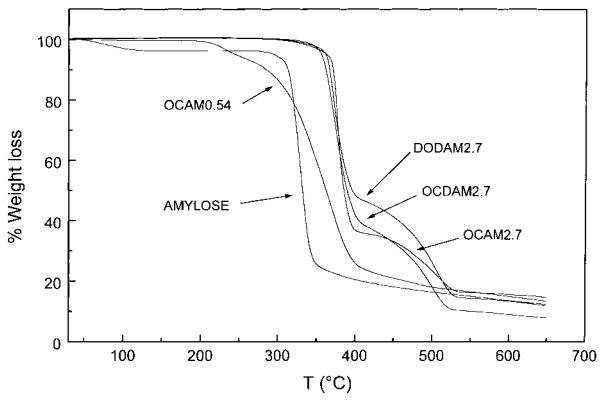


Figure 4 Thermal stability of amylose esters.

composition mechanism of starch is the dehydration reaction between starch molecules.[19] Thus, thermal stability increases with the degree of substitution since lower amounts of hydroxyl groups remain. Examining more carefully the decomposition thermograms of the esters, one can see that decomposition takes place in two stages. In the first stage, the weight loss is about 55–63% of the initial weight. The second stage starts above 400°C and ends at about 525°C, giving an ash residue. This second decomposition stage may be attributed to the methylene groups of the side esters since it does not appear in pure amylose. This stage is also similar to the decomposition of low-density polyethylene (LDPE) (which contains only methylene groups).

The internal plasticization provided by the bulky side ester groups has also a profound effect on their ability to form films, compared to pure starch or amylose, for which films are almost impossible to prepare. The film formation properties of the neat esters that were studied depend mainly on the degree of substitution and not on the length of the side group. The esters with high degree of substitution can more easily form flexible film. On the contrary, film of esters with a low degree of substitution can be prepared only with great difficulty and are very brittle. The above behavior is strongly reflected upon the mechanical properties of the esters, as can be seen in Table III.

It is evident that starch and amylose esters with a high degree of substitution behave-like typical thermoplastic materials, showing poor tensile strength and high elongation at break. The octanoated and dodecanoated starch or amylose esters with ds 2.7 have about the same tensile strength but the elongation at break increases with increasing side-chain length. Obviously, the bulkier groups are more effective internal plasticizers. The degree of substitution in the above esters plays also an important role in the final properties. Tensile strength decreases as the ds becomes higher, whereas the opposite trend is observed for the elongation at break. It seems that the replacement of only a small fraction of the hydroxyl groups cannot provide a drastic plasticization, and the final product retains some of the mechanical properties of the unmodified starch.

The picture is quite different in octadecanoated esters which behave more like brittle materials. Octadecanoated starch with lower degree of substitution (ds 1.8) has higher tensile strength and lower elongation at break than the above-mentioned esters. The elongation at break remains relatively low, even for high degrees of substitution. Such a trend was not observed in octanoated and dodecanoated esters. This sudden reversal in behavior must be attributed to the partial crystallization of C_{18} side chains, as demonstrated by DSC measurements. Crystalline materials usually show high tensile strength and low elongation at break. Thus, it can be said that there is an optimum side chain length, which provides an effective plasticization.

Comparing the corresponding starch and amylose esters, amylose esters have slightly higher tensile strength, as well as elongation at break. The native starch used contains mainly amylopectin, which is a branched macromolecule, and as such, it has a lower ability to form chain entanglements, compared to the linear amylose macromolecule. It has been found that cast amylose films are more flexible than films prepared by amylopectin.[20] This trend must apply to their esters as well. Indeed, it was found that it is easy to prepare amylose triacetate films but preparation of films from acetylated amylose–amylopectin mixtures containing more than 60% amylopectin is not possible, as the resulting films are too brittle.[21] Such a phenomenon was not observed in our starch esters, even though they contain a higher proportion of amylopectin (81%). Obviously, the long fatty side groups are more effective plasticizers than acetates.

The tensile strength of our starch and amylose esters is lower than those mentioned for destructurized starch, which lies between 20–30 MPa.[17,22] The lower values are due to the internal plasticization effect of the bulky fatty ester groups. Because of this plasticization, they have a higher elongation at break compared to that of extruded starch (about 4%). The mechanical properties of the above esters appeared to have similar behavior with starch plasticized with glycols and, especially glycerine, which acts as an external plasticizer. The properties of the plasticized starch depend on the glycol type as well as its concentration. In the case of starch and amylose esters, properties can be easily adjusted by changing the chain size and/or the degree of substitution. One of the advantages of polysaccharide esters is that the plasticizing groups are covalently bonded and cannot migrate. This leads to stable mechanical properties throughout the service life of the material. On the contrary, in plasticized starch, it is possible to have some loss of the

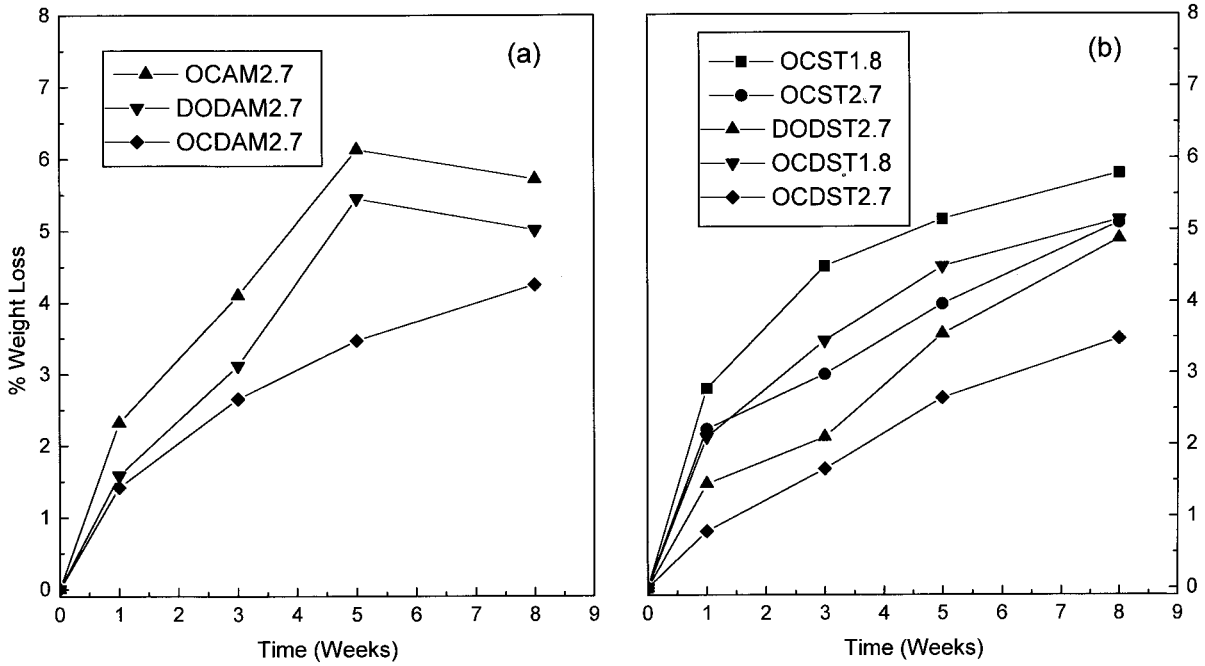


Figure 5 Weight loss (a) amylose and (b) native starch esters during exposure to activated sludge.

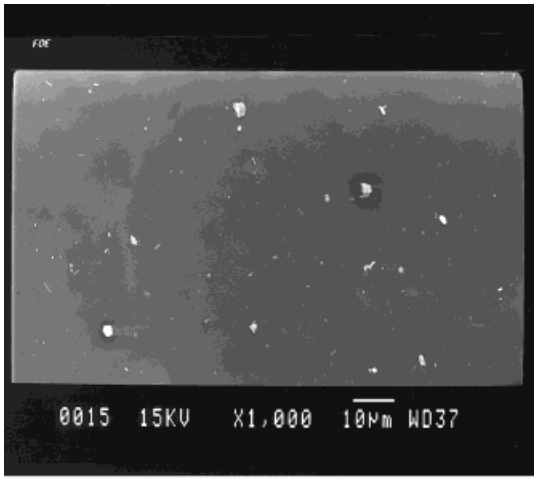
plasticizer molecules through migration or evaporation, which leads to an alteration of its mechanical properties.

Biodegradability

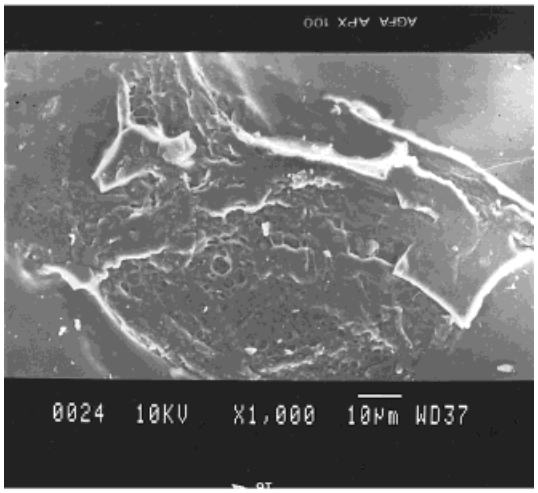
A critical issue concerning the usability of the newly synthesized esters is their biodegradability. After all, the production of new materials having no biodegradability, would be of little value. The biodegradation rate of the prepared esters was followed by determining their weight loss when they were exposed to activated sludge. The results of the biodegradation study are shown in Figure 5.

The biodegradability of the prepared esters appears to be rather low since the weight loss does not exceed 6% within the time period studied. The amylose esters show slightly higher biodegradability than their native starch counterparts, probably due to the linear character of the amylose molecule. It was observed by Bhattacharya et al.[23] that starch (with 70% amylose)–styrene maleic anhydride blends were a little more biodegradable than those containing only amylopectin. Comparing the different esters, it can be said that those with lower degrees of substitution show higher biodegradability. Octanoated amylose with low degree of substitution has the highest biodegradation rate. Similar findings have been reported for starch acetate.[11] It seems also that the biodegradation rate increases with shorter side-chain length. Obviously, the bulky groups introduced by esterification are interfering with the biodegradation process, possibly by inhibiting the catalytic action of amylases, which are responsible for the biodegradation of the starch.

The SEM photographs of the exposed samples seem to corroborate the findings from the weight loss measurements. Figure 6 shows the SEM photographs of an octanoated amylose sample with degree of substitution 2.7, before and after 3 weeks of exposure. The film surface is becoming progressively more rough as time passes due to starch removal. It is evident that there is some material consumption, but this happens only in small areas of the film surface and, thus, only a small weight loss was detected within this exposure time. More interestingly, microbial colonies are visible on some films. Starch removal seems more intense around these areas of increased microbial population. This is an indication that starch removal is mainly attributable to microbial activity, although there may be other secondary factors, such as mechanical abstraction and starch dissolution. The same behavior appears for



(a)



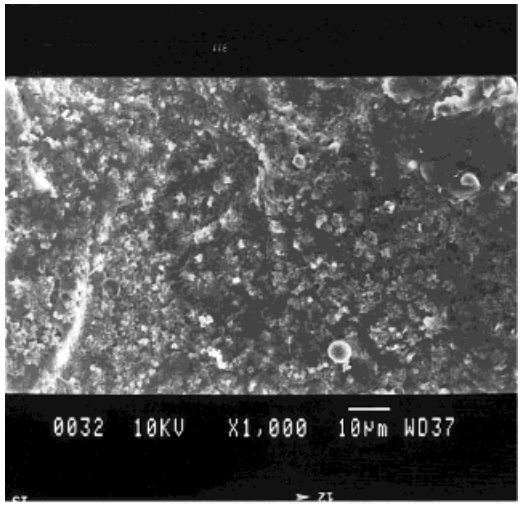
(b)

Figure 6 SEM photographs of an octanoated amylose sample after (a) 0 and (b) 3 weeks of exposure to activated sludge.

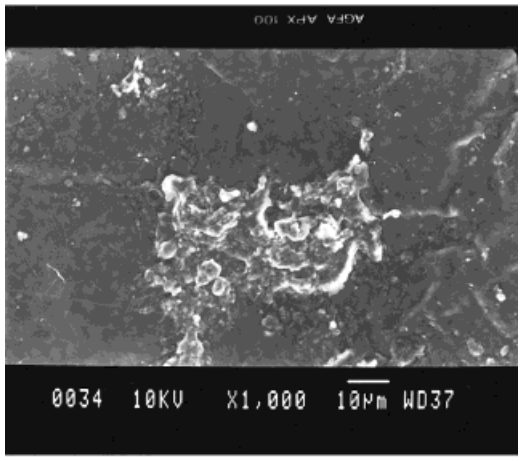
the other esters with high degree of substitution as well.

The effect of the degree of substitution is more clearly illustrated in Figure 7, which shows the SEM photographs of two samples of octadecanoated starches with degrees of substitution of 1.8 and 2.7, respectively, after 3 weeks of exposure to activated sludge. The modified starch with the lower degree of substitution is affected to a greater extent. This finding is in good agreement with the biodegradation results of octanoated starch esters during soil burial.[23]

The mechanical properties of biodegraded esters seem to corroborate the findings of weight loss measurements and SEM photographs. Tensile strength decreases only slightly with exposure time. It must be noted, however, that the tensile strength of the unexposed samples is already rather low, so the differences might not be so pronounced as they lie within experimental error. Only amylose C_{18}-ester shows a significant decrease (25%) in tensile strength (Fig. 8). This is probably due to the fact that this ester has a significant tensile strength (4 MPa) and the differences are more pronounced. In OCAM0.54, due to the high biodegradability and dissolution in water that take place simultaneously, it was impossible to measure the tensile strength after their exposure to activated sludge. The elongation



(a)



(b)

Figure 7 SEM photographs of (a) OCDST1.8 and (b) OCDST2.7 samples after 3 weeks of exposure to activated sludge.

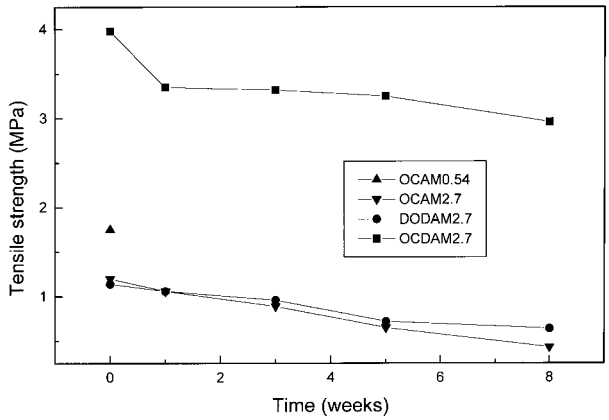


Figure 8 Tensile strength variation of amylose esters during exposure to activated sludge.

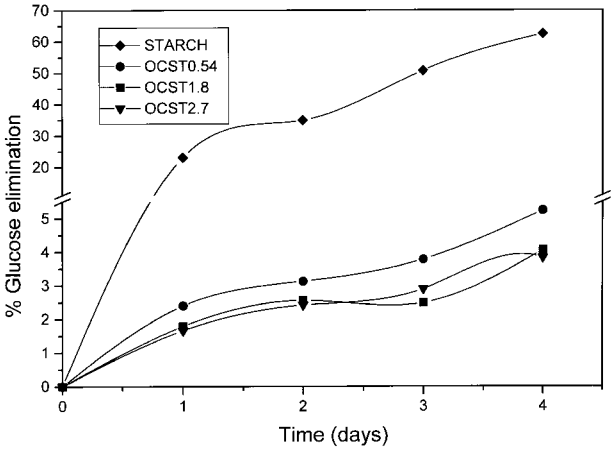


Figure 9 Percentage of consumption for various octanoated starches after exposure to α-amylase–lipase mixture ($C_{amylase}$ = 40 mg/mL; C_{lipase} = 4 mg/mL).

at break follows a similar trend. Again, there is a small reduction, especially in samples with high elongation at break. Most notably, the DODST2.7 ester shows a great decrease in elongation at break.

Bacteria-consuming starch are using enzymes, such as α,β-amylases, which act through a complex formation in an active site close to the ether bond formed between two α-D-glucopyranose groups and finally lead to its breaking.[24,25] Since most of these bonds are shielded in the starch esters by the bulky ester groups, the above complexes are more difficult to form. It must be noted, however, that many microorganisms also produce enzymes called esterases, which are able to break ester linkages. Thus, the actual biodegradation process may involve all the above-mentioned enzymes. To verify the above assumption, octanoated starches with different degrees of substitution (0.54, 1.8, and 2.7) were exposed to enzymatic hydrolysis using α-amylase–lipase mixtures with different lipase contents. The results are shown in Figures 9 and 10.

Pure starch seems to be easily hydrolyzed irrespective of the environment. Esterified starches, however, show only limited hydrolysis, in agreement with weight loss measurements previously discussed. Samples exposed to a higher concentration of lipase show a greater extent of hydrolysis (Fig. 10). Also, the differences between the esters with different degrees of substitution are more visible than in the case where only 4 mg/mL lipase were used. This proves that the presence of lipase has a beneficial effect on hydrolysis because it leads to a cleavage of the ester groups and permits easier attack by α-amylase. The enzymatic hydrolysis experiments further confirm that the degradability of starch reduces with increasing degree of substitution, as it was found from the exposure to activated sludge.

CONCLUSIONS

A series of systematically hydrophobized starch and amylose esters have been prepared and studied. In these modified polysaccharides, the hydrophobicity and water absorption is increasing with the degree of substitution and side-chain length. The mechanical properties of the esters also depend on the degree of substitution. Those with

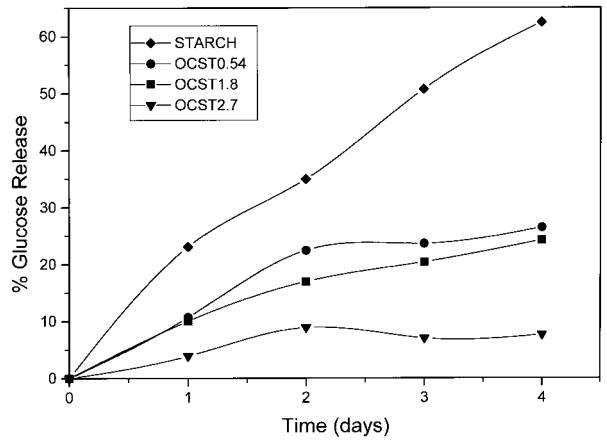


Figure 10 Percentage of consumption for various octanoated starches after exposure to α-amylase–lipase mixture ($C_{amylase}$ = 40 mg/mL; C_{lipase} = 40 mg/mL).

high degree of substitution show good elongation properties but rather low tensile strength. By changing the fatty chain and the degree of substitution, materials with a wide range of properties can be prepared. As a result of their hydrophobic character and permanent internal plasticization, these materials are expected to show a stability in their properties throughout their service life.

The prepared esters are biodegradable, and their biodegradability depends mainly on the degree of substitution and the side-chain length. The biodegradability of these materials, however, appears to be rather low, compared to pure starch, especially for those with a high degree of substitution. Longer exposure times are needed in order to determine their long-term biodegradability. The biodegradation results of the prepared esters during exposure to activated sludge are in good agreement with those of enzymatic hydrolysis. Lipase can increase the biodegradation rate of the prepared esters.

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