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Thermal characterization of starch-based polymers produced by *Ophiostoma* spp

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Abstract The thermal behavior of modified starches (MS) produced by biosynthetic pathway is described based on a comparative analysis with native starches (NS). MS were produced by fermentation in presence of *Ophiostoma* spp. cultures. Thermogravimetric analysis (TG) with successive derivatives (DTG) and differential scanning calorimetry (DSC) were used for this study. NS results showed a single peak dominating both the TG (DTG) and DSC plots. A double thermal transition event was detected in samples of MS. The procedural decomposition temperature (T_i–T_f; lowest onset temperature of initial and final mass change) was carried out within a narrow interval of temperatures for NS (610–640 °C). This interval could not be reached within the 1,000 °C range in MS. Residues higher than 10% were recorded for MS at this temperature. The presence of the double thermal transition in MS is discussed.

Keywords *Ophiostoma* spp. · Starch modifications · Modified and native starches thermal analysis

Introduction

TG and successive DTG have been used before to study the mechanisms of degradation of starches or their derivatives. Although, just one-reaction process has been observed in the different starches, it has been shown that different starch sources can be readily differentiated by the comparison of their respective decomposition temperature intervals; T_i–T_f, where T_i is the lowest temperature at which the onset of a mass change can be detected for a given set of experimental conditions and T_f is the lowest temperature at which the onset of the mass change has been completed [1].

DSC has been extensively used to study the T_m (melting point) on starch granules and T_g (glass transition temperature) on amorphous materials. Many authors have reported results with wide variations which could be attributed to the measurement conditions and to the complex behavior of starch and starch preparation or physical morphology. Results are entirely dependent on the moisture content (mc) (or plasticizer), T_m is thermodynamically irreversible and it is not well differentiated from the T_g, i.e., waxy maize tested in aluminum pans at ~13% mc presented an endothermic heat flow at ~60 °C with onset at ~40 °C. This peak was considered as a change of heat capacity associated with the T_g. When this sample was tightly packed in stainless steel O-ring pans by ultra-sound bath the thermal plot showed a double transition event with peaks at 170 and 190 °C (the heat conductivity can be also increased with salts, such as KCl). O-ring pans loaded with ~26 mg of sample at ~56% water content the observed endothermic transitions were 4 at ~70, 90, 110 and 130 °C. The origin of these peaks was not explained by the respective authors [2]. By the other hand, rice starch tested in aluminum sample pans at ~60% mc produce a strong peak at ~70 °C which was related to the gelatinization temperature of the starch (onset temperature ~60 °C and conclusion temperature 90 °C) [3]. Two peaks in waxy wheat found at ~65 and 105 °C (starch tested at mc of 50% in aluminum pans) were related to the starch gelatinization and the dissociation of amylose-lipid complexes respectively [4]. Blends of different starches do exhibit two different heat flow transitions [5]. Gelatinized starches

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exhibit also just one endothermic peak associated to T_g [6]. Gelatinization is a complex process which includes the disruption of the crystalline regions (T_m) within the granules, while the phenomenon of the T_g occurs just to amorphous material. The differences in temperatures for the endothermic peaks among different starch samples are associated therefore to the starch composition (amylase to amylopectin ratio), granular architecture (crystalline to amorphous ratio) and M_w (molecular mass) as well as polydisperisty of the chains [3, 7–10]. The chemical composition and granular architecture influences also starch derivatives.

This study was undertaken to assess thermal differences between native starches and granular modified starches (Bio-Carb) produced by the methods exposed by Sain et al. and Jeng et al. [11, 12]. These materials are being produced in culture media in presence of Ascomycetes fungi. Huang et al. [13] described some of their physical as well as mechanical properties and discussed their potential applications of these materials for the packaging industry. In specific they reported better film formation, higher mechanical properties and lower water adsorption compared to native starches. Their results led them to conclude that the process of synthesis was related to important structural modifications of the starch molecules. However, there is a lack of chemical information related to these materials. The main objective of this study was to clarify whether these modifications are taking place or how these modifications may influence the starch functionality. The samples were analyzed by thermogravimetry (TG), derivative thermogravimetry (TG) and DSC. TG curves were not interpreted in terms of the exact reactions occurring during the modification. Instead, such plots were used as a 'fingerprint' for differentiation purposes to show the impact of such modification over the starch molecules.

Materials and methods

Production of starch-like polymers and preparation of films for thermal analyses

The starches in this study were from commercial sources (type A). The starch conversion was carried out in culture media with *Ophiostoma* spp. cultures according to the methods followed by Jeng et al. and others [11–14]. The absence of protein was confirmed from free cell starch-polymer samples by using crystallized bovine albumin (Sigma Co., U.S.A.) as standard and Coomassive brilliant blue R-250 as the dye reagent (method from Bio-Rad Bradfor Protein Assay with BSA; bovine serum albumin) at a UV adsorption of 595 nm. The measured proportion of protein was ~0.019%. Therefore, relatively pure materials were produced for further analyses. Two different samples were used for these analyses: in powder form and films. Powders were used for TG and TGA. Powder and films were used for the DSC analyses. The films were prepared by the casting method as shown by Myllarinen et al. [15]. Roughly, the film-forming solutions were prepared by pouring the starch in deionised water while stirring for 15 min. The solutions were spread onto polystyrene Petri dishes (15 cm diameter) and cooled at room temperature. The air bubbles were removed by vacuum. Film thickness was controlled by using the same amount of material based on starch mass per plate. Samples of native as well as modified starches in both forms; powders and films, were conditioned at 20 °C and 65% RH (relative humidity) for mass stabilization before analyses.

TG and DSC apparatus and sample preparation

The equipment of the thermal analysis consisted in a TG-DTG (thermo-gravimetry-differential thermal analysis) unit from TA instruments, model Q500. The integrated software provided the TG and successive DTG derivative signals of the samples which were thermally treated. The purge gas flow rate was settled at 100 ml min^{-1}. Rising temperature experiments were conducted in which the heating rate was 5 °C/min for all experiments. With the aim of avoiding the oxidation of the species, the thermal decomposition was performed in an atmosphere of flowing nitrogen at 100 ml min^{-1}. Temperatures were risen up to 1,000 °C.

Differential scanning calorimetry (DSC) curves were acquired to study the course profile of the gelatinization process in both starches; modified and unmodified, in presence of KCl to increase thermal conductivity in the starch samples. DSC was performed with a Q1000 unit from TA Instruments fitted with a cooler system and nitrogen gas as purge. All samples were tested by triplicate in aluminum pans with 30 µl volume capacity-PE No.-BO169320, and empty pan as reference. Samples were made up as starch slurries. The maximum temperature reached 250 °C at a heating rate of 5 °C/min, with size samples of 5–7 mg. DSC data parameters collected with the software integrated (TA Instruments; Universal Analysis) included: onset temperature (T_{ON}), maximum peak at melting point (T_M), final melting temperature (T_C), melting temperature interval or the start and conclusion temperatures (T_R) and change heat capacity (C_p) at the measured melting point. T_R is defined as the point at which the DSC trace line first ceases in following a straight line and finally recovers the base line. The onset temperature was taken at the first inflexion point on the trace curve line of the DSC endotherm. The maximum endothermic peak temperature is normally used for the determination of the enthalpy of gelatinization.

Results and discussion

TG

TG curves and respective first derivatives of mass against temperature (%/ °C) (DTG) for native as well as modified starches at 1,000 °C are shown in Figs. 1 and 2. The curve of mass loss against temperature showed a single-stage decomposition temperatures (T_{i}–T_{f}) in both samples of NS and MS. DTG plots of modified starches showed at this zone of mass/loss transition the maximum temperature of ~307 °C; value which was slightly higher in native starches ~318 °C. The percentages of residual mass recorded at these temperatures were ~40% and ~50% for NS and MS respectively. The mass loss in MS did not go to 100% even at temperatures above 1,000 °C. Instead, remaining masses higher than 10% were recorded for different samples of MS, including modified tapioca (branching type C), potato (type B) or corn (type A) starches. In contrast, the plot in Fig. 1 shows the complete degradation of native starches at ~600 °C. It was deduced at this point that some heat resistant chemical species are being formed in the modified starches. Some examples of such materials potentially formed include carbonaceous char- or graphite-like residues or ionic structures which can derive inorganic compounds during the process of degradation. For some samples of MS it was found a slight gain in mass at temperatures over the 1,000 °C (in air at elevated temperatures a polymer will eventually oxidize with subsequent chain scission and degradation giving rise purely to a 100% gaseous mass, the mass gain during the combustion in presence of air is an erroneous result, due to the formation of oxide-like compounds).

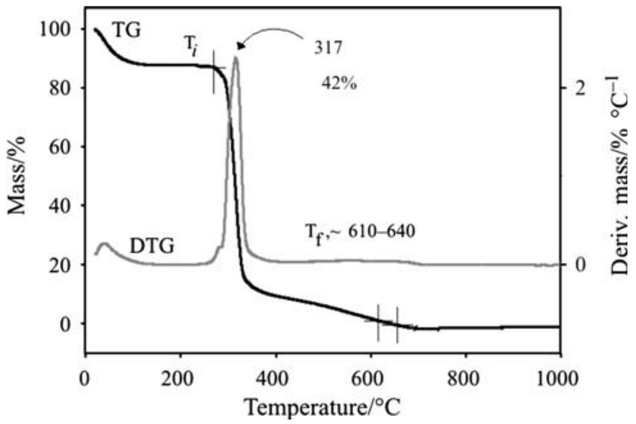

Fig. 1 TG-DTG plot of NS

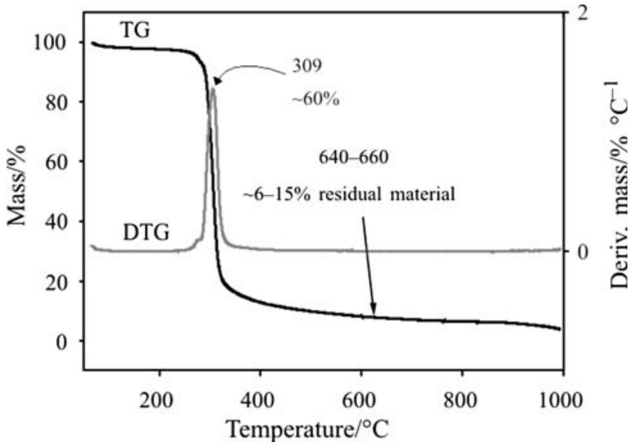

Fig. 2 TG-DTG plot of MS

A plot of time against temperature appears always as a straight line with the slope of the curve indicating the heating rate employed. The variations due to the reaction of the material under increasing temperatures cannot be easily appreciated due that the instrument adjusts automatically the heating rate. However, differences in exothermic or endothermic heat capacity respect to the constant heating rate can be observed in successive derivatives of mass [%/°C and 2nd derivative %/°C^2] and times [min/°C and min/°C^2] versus temperature (°C).

The rate of mass change versus the temperature is shown in Fig. 3 (1st and 2nd derivatives of mass versus the temperature). The %/°C^2 displayed the minute separation of two endothermic peak events not observable in native starches. Successive Derivatives of time versus the temperature (min/°C and min/°C^2) for modified starches as well as native starches are shown in Figs. 4 and 5. The maximum fluctuations were observed in samples of modified starches.

The first stage of discontinuity observed before the 100 °C in the TG plots of native starches was related to the loss of water. The loss of water in a first stage of the heating was not observed in modified starches. For some samples of modified starches (not shown in Figures), the

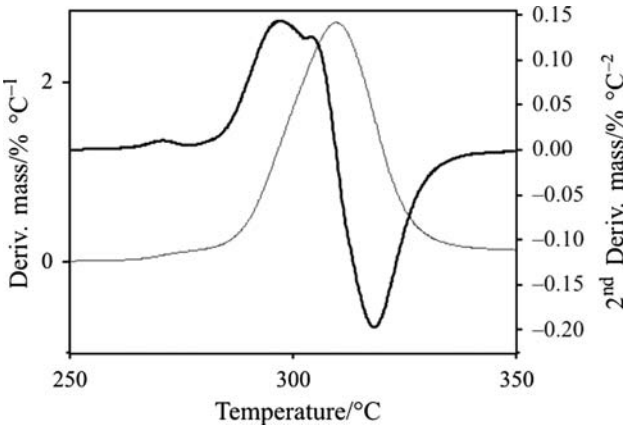

Fig. 3 Successive derivatives in MS shown in mass percentage

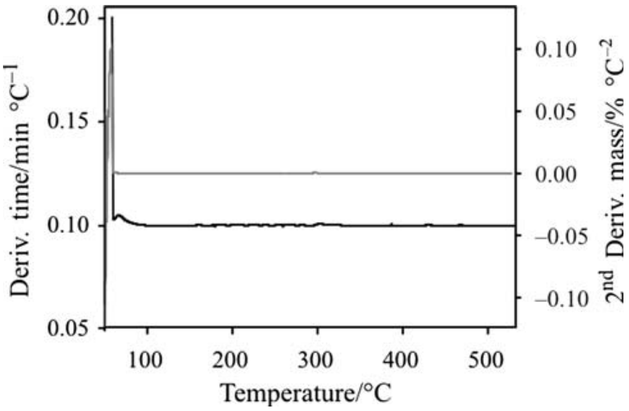

Fig. 4 Successive derivatives MS shown in time

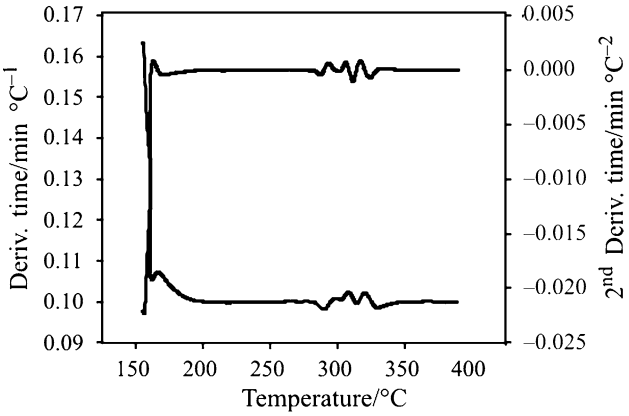

Fig. 5 Successive derivatives NS shown in time

lack of phasing respecting the initial mass loss was probably due to the environment of the decomposition (i.e., the presence of volatile products of decomposition in the sample which probably induced their initial behavior).

DSC

DSC parameters are shown in Table 1: Onset (T_{ON}), maximum peak at melting point (T_M), final melting temperature (T_C), melting temperature interval (T_R), heat capacity (C_p); the enthalpy of fusion (ΔH_{fus}) can be deduced from C_p at the recorded temperatures shown in the Table. The main differences may be observed from modified starches in powder form respecting to the rest of the samples. However, one of the most interesting results was recorded from films manufactured with modified starches. Before film formation the T_R was $\sim$64 and after the manufacture of the films this value was lower than films manufactured with native starches suggesting a better molecular packing during film formation. Similar effect was found for T_{ON}, T_M, T_C and the maximum endothermic heat flow. The C_p was as low as 8.2 J/(g °C) in modified starches in powder form, however, after film formation this value is similar to that found for films manufactured with starch granules being of $\sim$70 J/(g °C). Similar results were consequently observed with the respective melting temperatures. The melting temperatures of granular native starches and films did not show such a sensitive variation. It is possible to infer a high degree of disturbance after the modification, however, the film formation showed a high level of molecular organization comparable to native starches.

Figures 6 and 7 show the resulted endothermic curve profiles for native as well as modified starches in powder form gelatinized with a solution 0.6 M of KCl. Native starches presented a narrower endothermic peak with a maximum temperature of $\sim$108 °C, while modified starches displayed the two peaks at $\sim$74 and 84 °C. Onset transition temperatures were found in $\sim$36 °C in modified starches while this same value was located at around 103 °C in unmodified starches. The peak at the maximum endothermic heat flow also for modified starches was observed at lower intensity. In presence of DMSO modified starch samples also slightly displayed two maximum peak transition points at $\sim$73.57 and 81.20 °C. The thermal transitions were smother comparer with starches gelatinized in KCl and the melting temperature interval was broader.

The endothermic curves profiles obtained from films manufactured with modified as well as unmodified starches

Table 1 DSC parameters: Onset (T_{ON}), maximum peak at melting point (T_M), final melting temperature (T_C), melting temperature interval (T_R), heat capacity (C_p)

Samples	DSC melting parameters				
	T_R/°C	T_{ON}/°C	T_M/°C	T_C/°C	C_p [kJ/kg °C] measured at the respective melting temperatures in °C
Modified starches in KCl (powder form)	64.2	35.9	74.9 83.8	100.2	$\sim$8.2
Unmodified starches in KCl (granular form)	17.4	103.1	108.6	120.4	$\sim$23.4
Modified starch films in KCl	16.4	103.7	107.4 108.2	120.2	$\sim$68.4 $\sim$66.7
Unmodified starch films in KCl	18.5	101.5	106.7	120.9	$\sim$72.3

and analyzed in presence of 0.6 M KCl are shown in Figs. 8 and 9. The endothermal course profiles recorded on films manufactured with native starches displayed an abrupt transition at ~102 °C, which is also the onset temperature for this sample, while the films from modified starches showed a smoother transition at the onset value of ~103 °C. Again, the temperature course profile in the case of modified starches showed the two thermal transition peaks at the tip of the curve with very close temperatures.

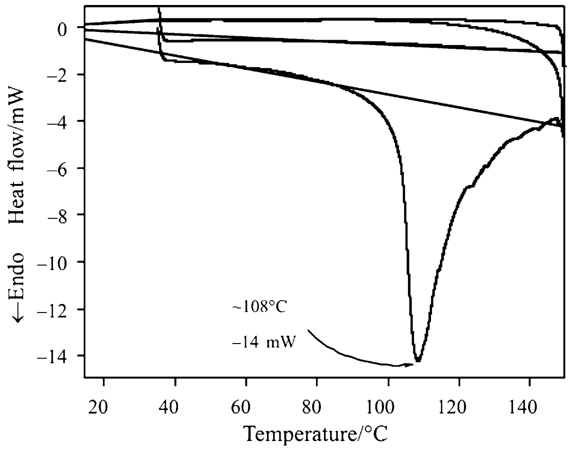

Fig. 6 NS in 0.6 M KCl (granular starch)

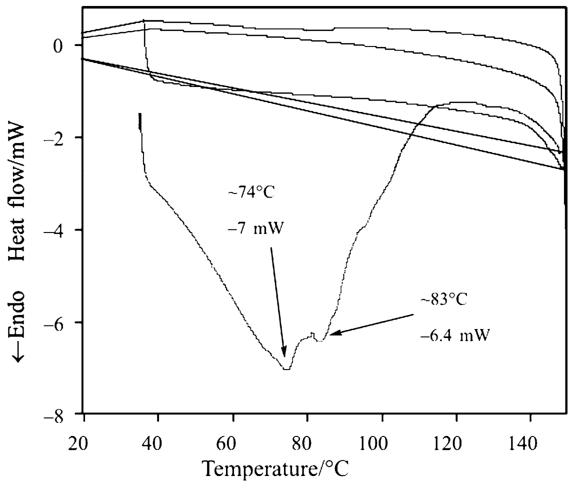

Fig. 7 MS in 0.6 M KCl (powder form)

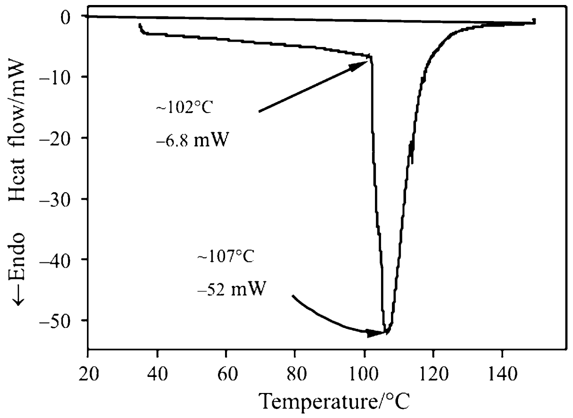

Fig. 8 NS films in KCl

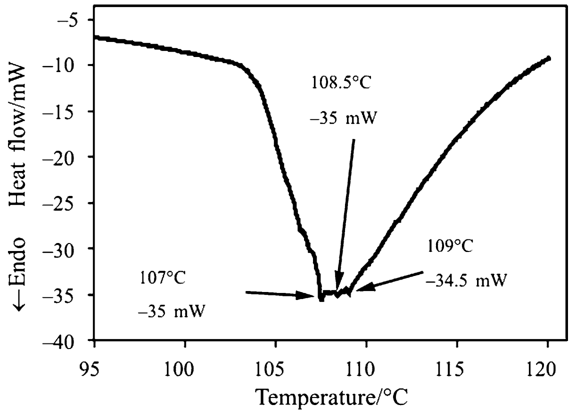

Fig. 9 MS films in KCl

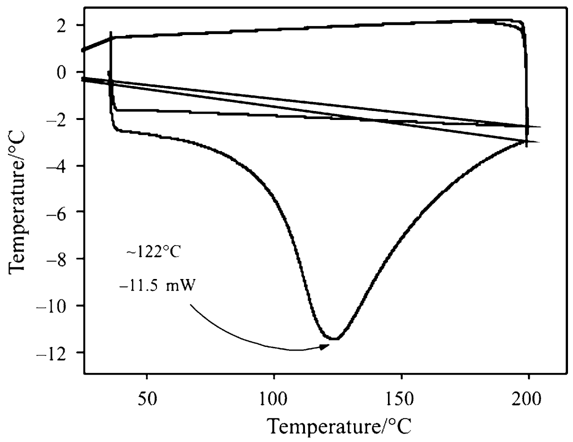

Fig. 10 NS films

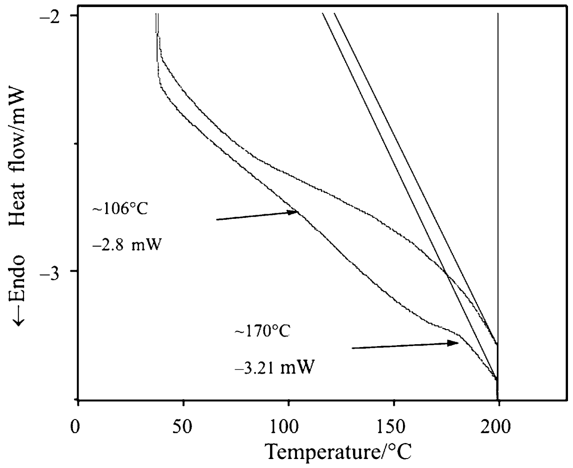

Fig. 11 MS films

The water loss measured by DSC in MS and NS films is shown in Figs. 10 and 11. The films were conditioned at constant mass at laboratory conditions (65% RH, 20 °C) for 3 weeks. The water bending curve due to water evaporation recorded for MS was much lower compared to films fabricated with NS. In general modified starches tend to adsorb less water at the same conditions of relative humidity.

Conclusions

Native and modified starches obtained by a biosynthetic route were treated to thermal analyses and the data compared. Modified starches showed a double endothermic peak transition; in both samples: powders form and after film forming, which can be associated to the presence of two different T_g transitions. It is possible to infer from the results a high degree of molecular disturbance after the modification and before film forming, however, films manufactured with modified starches showed a high level of organization comparable to native starches. DSC analyses for these modified starch films showed two endothermic events which were associated again to the T_g transitions. If the ordered regions of a polymer are judged by differences in the melting heat flow profiles, these new polymers had less ordered arrangement when obtained in a powdery form (after the modification), but present a better molecular packing after film forming. Such molecular organization can reduce also the atmospheric water adsorption. In relation with these arguments, the experiments also showed low water adsorption in modified starches. For starch based materials the atmospheric water intake is strongly linked to their functionality and potential applications, the reduction of atmospheric water intake by any means constitutes a substantive starch improvement. Comparable heat capacities between native and modified starches suggest also the presence of polymers of high molecular mass or the lag (null) degradation of the starch molecules. TG analysis confirmed the presence of a double signal together with the potential presence of materials withstanding temperatures up to 1,000 °C and higher.

Data reported by Recently Sain et al. (op. cit.), Jeng et al. (op. cit.), Huang et al. (op. cit.) and Sain and Jeng [16] based on the mechanical testing of films prepared with MS show a better film formation, higher mechanical properties and lower water adsorption compared to NS. Their results are linked to structural modifications of the starch molecules occurring during the enzymatic modification. *Ophiostoma* spp. is known to produce extra cellular polysaccharides (EPS) and various enzymes which have misleading pathogenetic activities when found in the living tree tissues [17, 18]. And there is a less marked relation with the host's storage tissues, mainly because the starch concentrations in the trees in which these fungi grow is relatively low. However, it is interesting to note that Binz and Canevascini [19] reported an exponential increase of β-glucosidase in presence of starch, while Jeng et al. (op. cit.) found that such modified starch polymers were partially hydrolyzed by this same enzyme. These arguments lead to the thesis that such microorganisms produce in a first stage of the modification a starch sub-product which is further utilized by the microorganisms for other purposes. This phenomenon can be supported with the results presented by Huang et al. (op. cit.) in which the mechanical properties as well as the molecular mass of the polymers when plotted against the time of modification showed a Gaussian curve with a maximum at the third day of modification. This result suggested the increase in the molecular mass of the material after the modification of the starch.

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