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STRUCTURAL INVESTIGATION OF SOME NATIVE BIODEGRADABLE PACKAGING MATERIALS

**Onuc COZAR[1]*, Nicolae CIOICA[2], Elena Mihaela NAGY[2],
Constantin COŢA[2], Radu FECHETE[3], Claudiu FILIP[4]**

[1]Babes-Bolyai University, Dept. of Physics, Kogalniceanu 1, Cluj-Napoca, Romania
[2]National Institute of Research-Development for Machines and Installations Designed to Agriculture and Food Industry – INMA Bucureşti – Cluj-Napoca Branch, RO-400458 Cluj-Napoca, Romania
[3]Technical University of Cluj-Napoca, Dept. of Physics, RO-Cluj-Napoca, Romania
[4]National Institute for Research and Development of Isotopic and Molecular Technologies, Donath no. 71-103, P.O. 700, 400293, Cluj-Napoca, Romania
**Corresponding author: onuc.cozar@phys.ubbcluj.ro*

ABSTRACT. The IR, Raman and NMR methods are used to investigate the structural effects of water and glycerol (plasticizers) on biodegradable packaging materials proceeding from native corn starch. The IR and Raman methods not give a clear response on the amorphous/crystalline content in the investigated starch samples because the origin of vibrational bands is in the same atomic groups of the major starch components (amylose, amylopectin). The nuclear magnetic relaxation data show that the amorphous/crystalline ratio depends not only by the amylose/amylopectin content but also of the mobility and the place of theirs polymer chain segments. The shapes of ^{13}C CP/MAS NMR spectra show clearly the presence of A, B, V-types crystalline structures and also amorphous phase which prevails with the increasing of plasticizers content and also in the extruded starch samples. The composition having starch/glycerol/water 68/17/15 mass % ratio was found to have a dominant amorphous character and very similar features with a commercial specimen (USA) used for the package. It was also found that this best package is extremely degraded after just one day under water absorption.

Key words: *starch, plasticizers, IR, Raman, NMR*

INTRODUCTION

The native starch is used as the main component of biodegradable packaging materials and also in the textile, pharmacological and the food industry (Almeida et al., 2010; Liu et al., 2009; Luckachan and Pillai, 2011). It has a granular structure with an internal architecture characterized by concentric rings involving semi-crystalline shell (thickness 120-400 nm) separated by essentially amorphous regions (Wang et al., 2009). Chemically, the starch contains two glucose polymers amylose and amylopectin (Liu et al., 2006).

Amylose is essentially a linear structure of α -1,4 linked glucose units, and amylopectin is a highly branched structure of short α -1,4 chains linked by α -1,6 bonds (Liu et al., 2009). Amylose and the branching points of amylopectin form the amorphous regions in the starch granules. Amylopectin is the main crystalline component in granular starch (Soesta and Vliegenthartb, 1997). The physical-chemical properties of starch as viscosity, gelation power, adhesion etc., are strongly influenced by the amylose/amylopectin ratio (Almeida et al., 2010; Cereda et al., 2002).

The plasticizers molecule (water, glycerol) and other proceedings as annealing extrusion or enzymatic digestion can destabilize the crystalline regions and increase the amorphous domains (Brümme et al., 2002; Lopez-Rubio et al., 2008; Warren et al., 2016).

There are four types of crystalline structures in starch materials. From these the A-type is due to the junction of amylopectin short branches to backbone chain (lowest mobility) close to each other and appears in corn, wheat or rice cereals. In fruit and tuber starches prevails the B-type structure realized by amylopectin polymer chain segments located away from junctions in core branches region (more mobile). The V-type structure is formed by inclusion complexes between amylose and water, glycerol, alcohols molecules (plasticizers) stabilized by hydrogen bonds. There is also a C-type crystalline structure in leguminous plant, which is in fact a mixture of A and B-type structures (Cioica et al., 2013). Amorphous phase is due to mobile laterale branches of amylopectin and free chains of amylose.

The native starch materials used in the packaging process must to have a high content of amorphous phase and biodegradability at natural agents (Luckachan and Pillai, 2011). Besides the presence of plasticizers generally is also used the extrusion process what disrupts and transforms the semi-crystalline structure of starch granules in a homogeneous amorphous material with increased biodegradability (Mościcki et al., 2012; Nabar, 2006). The effect of the nature and content of the plasticizers (water, glycerol) on the native corn starch properties (crystalline/amorphous) and also on the degradation process after absorption of distilled water were investigated by vibrational (IR, Raman) and nuclear magnetic resonance (NMR relaxation, ^{13}C CP/MAS NMR) spectroscopies.

EXPERIMENTAL

The native corn starch used in this study was obtained from SC Amylon Sibiu, Romania, having water content of 10.76 %, a density of 0.561 g/cm^3 and an amylose content of 21%. The glycerol used was purchased from SC Nordic Invest SRL Cluj-Napoca and had a concentration of 99.5% and a density of 1.262 g/cm^3. The water used was from the water supply system.

Two sets of samples with different starch / glycerol / water [%] ratios were prepared. First set of samples have the ratio starch / glycerol (= 4) constant and the added water content increased from 0 up to 15% (0; 2.5; 5; 7.5; 10; 12.5; 15). Samples from the second set have constant the ratio starch / water (= 5.7) and the glycerol content increases from 0 up to 16.5% (0; 2.5; 6; 9.5; 13; 16.5).

FT-IR/ATR spectra were recorded at room temperature on a conventional Equinox 55 (Bruker, Germany) spectrometer equipped with a DTGC detector, coupled with an ATR sampling device (Miracle, Pike Techn.). The resolution was of 2 cm^{-1}. The ATR crystal is of ZnSe and we made 40 acquisition for obtaining IR spectra.

FT-Raman spectra were recorded with a resolution of 4 cm^{-}1 in a backscattering geometry with a Bruker FRA 106/S Raman accessory equipped with a nitrogen cooled Ge detector. The 1064nm Nd:YAG laser was used as excitation source, the laser power measured at the sample position was 300mW.

The ^{1}H NMR relaxation measurements were obtained using the Bruker Minispec spectrometer with the 10 mm probe-head working at 19.688 MHz Larmor frequency. In all measurements the temperature was set at 35 °C. The CPMG (Car – Purcell – Meiboon – Gill) decays and saturation recovery curves were analyzed using the UPIN (Uniform – Penalty Inversion) algorithm, which perform a Laplace inversion of the measured data (Borgia et al., 1998). In this way the T_1 (spin-lattice) and T_2 (spin-spin) relaxation times distributions as function of samples composition were analyzed.

The ^{13}C solid state cross-polarization magic-angle-spinning (CP/MAS) NMR spectra were recorded on a Bruker Avance III wide-bore spectrometer operating at 125 MHz Larmor frequency for ^{13}C, using a 4 mm double resonance probe head. Standard ^{13}C CP/MAS experiments were analyzed at room temperature, using a spinning frequency of 14 kHz and a ^{1}H 90° pulse of 2.3 μs. The ^{13}C CP/MAS spectra were calibrated using the $^{13}CH_3$ line in TMS through an indirect procedure which uses the carboxyl NMR line in α-glycine.

RESULTS AND DISCUSSION

Vibrational spectra

The characteristic IR spectra of starch samples with different amounts of plasticizer agents (water, glycerol) are situated in the 2900 cm^{-1}, 1340 cm^{-1} and 1000 cm^{-1} regions. These spectra contain also two bands at 3300 cm^{-1} and 1610 cm^{-1} due to the water stretching and bending vibrations (Morales et al., 2012; Iizuka and Aishima, 1999).

The absorptions situated at 2930 cm^{-1} and 2850 cm^{-1} are assigned to symmetric and asymmetric stretching vibrations of CH_2 groups [16] and those from 1350 cm^{-1} region to bending modes of O-C-H, C-C-H and C-O-H angles of amylose and amylopectin formations (Bellon–Maurel et al., 1995).

The peaks from the 1150 – 900 cm^{-1} region are due to C-C and C-O stretching vibrations (Wilson and Belton, 1988).

According to Capron et al., 2007, the intense absorption from 998 cm^{-1} may be assigned to crystalline domains whereas the band at 1015 cm^{-1} reveals the amorphous contribution of plasticizers in starch samples (figure 1A).

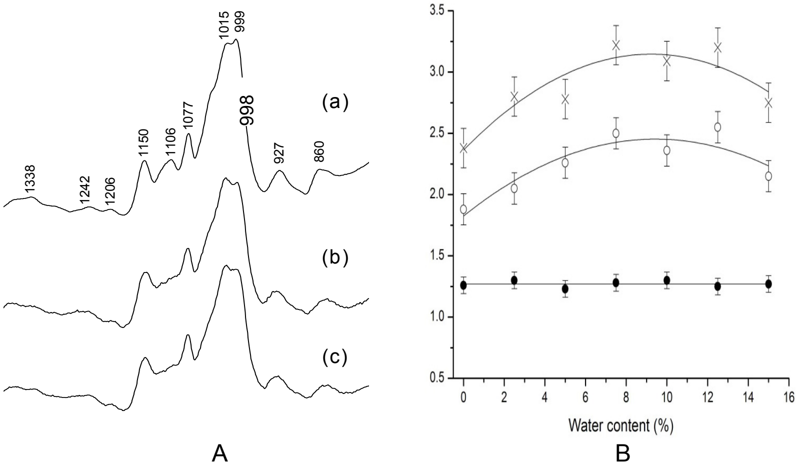

Fig. 1. *A. - The FT-IR spectra (in cm^{-1}) of starch / glycerol / water = 68/17/15 mass% sample before (a) and after extrusion (b) and of commercial specimen – USA(c). B - The dependence of 998 / 2929(x), 1015/2929(o) and 998/1015(•) ratios band intensities versus water content*

The evolution of the 998 / 2929 and 1015 / 2929 band intensity ratios versus water content (0 – 15 %) at a constant starch: glycerol ratio of 4 : 1 suggest a similar behavior (a parallelism) between increase and decrease of the crystalline / amorphous domains in the investigated starch samples with various plasticizers contents (figure 1B).

The constancy of 998 / 1015 ratio with the increase of water content suggests an absence of structural order at the FTIR observation range. A similar situation is also mentioned for the 1000 / 1022 cm^{-1} ratio in the case of extruded starch and granular starch with water content under 20% (Capron et al., 2007).

The band from 1015 cm^{-1} becomes more intense than that situated at 998 cm^{-1} in the extruded sample (figure 1A, b, c) being similar with a commercial specimen (USA). The extrusion process made with a co-rotating intermeshing twin-screw extruder ZK 25 (Collin, Germany), disrupts and transforms the semi-crystalline structure of starch granules in a homogeneous amorphous material which increase their biodegradability.

Raman spectra contain the two intense bands at ~ 2900 cm^{-1} attributed to the symmetrical and antisymmetrical CH_2 stretching vibrations (Almeida et al., 2010; Morales et al., 2012). Their intensity can be related to the amount of amylose and amylopectin content in the starch samples (Kizil et al., 2002).

The 1461 cm^{-1} band corresponds to the superposition of CH, CH_2 and C-O-H bending vibrations (Benzerdjeb et al., 2007) and those from 1380 cm^{-1} and 1340 cm^{-1} are due to C-O-H deformation modes and to the superposition of C-O stretching and C-O-H deformations, respectively (Almeida et al., 2010; Gussem et al., 2005).

The 1200 cm^{-1} - 1000 cm^{-1} region is characteristic of the C-O and C-C stretching and C-O-H deformation modes (Nikonenko et al., 2005; Yang and Zhang, 2009).

The bands observed at 940 cm^{-1} and 860 cm^{-1} are due to the superposition of bending C-O-C, C-O-H and stretching C-O vibrations from amylose α-1,4 glycosidic linkage (Almeida et al., 2010) and also to the superposition of C-C-H and C-O-C deformations, respectively (Gussem et al., 2005).

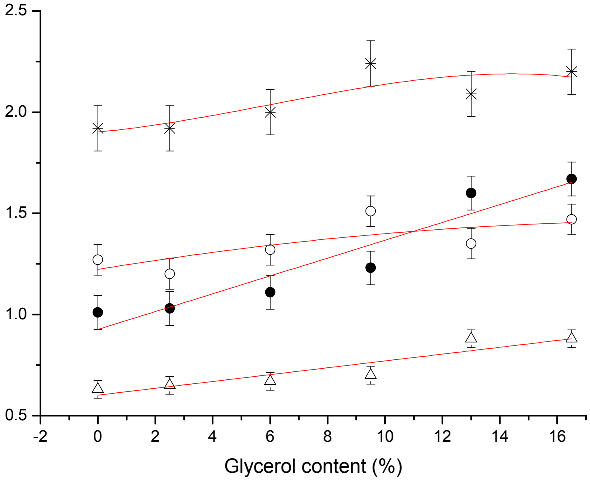

Fig. 2. *The dependence of 2900 / 1123 (∗), 1460 / 1340(•), 2900/477 (o) and 869 / 940 (Δ) ratios band intensities versus glycerol content and starch / water ratio constant (5.7). The errors bars are under 5% error limits*

An other intense 477 cm^{-1} Raman band is due to the superposition of bending C-C-C and twisting C-O vibrations and is used as a marker to identify the presence of starch in different samples.

The glycerol (OH · CH$_2$ · CH(OH) · CH$_2$ · OH) stretching ν_s, ν_{as} – CH$_2$ vibrations, the in plane δ -CH$_2$ deformations and CC stretching vibrations contribute to the following starch bands: 2900 cm^{-1}, 1461 cm^{-1} and 860 cm^{-1}, respectively.

An easy increase of the 2900/1123 (*), 2900/477 (o) and 868/940 (Δ) ratios band intensities may be observed for the samples, with starch / water ratio of (5.7) and the added glycerol increasing up to 16.5% (figure 2). These suggest that the amylose / amylopectin ratio remains practically unchanged and plasticizing – antiplasticizing effect depends on the levels of realized hydrogen bondings which lead to decrease - increase the rigidity of starch components.

The more pronounced increase of the 1460/1340 (•) band ratio is due to the glycerol contribution at 1461 cm^{-1} band by the in plane bending CH$_2$ vibrations from the inclusion complex realized between amylose and glycerol molecules by hydrogen bonds (V – type structure).

NMR data

The nuclear magnetic relaxation method (Cioica et al., 2013) offer information's on the structure of monomer units by direct spin–spin interactions (T_2) and also on the supramolecular organization by spin diffusion, spin–lattice relaxation (T_1).

A number of three peaks, two intense at T_2 ~ 0.2 ms and T_2 ~ 1 ms and also a small peak at T_2 ~20 ms are observed for pure starch and for a mixture of starch with water (figure 3).

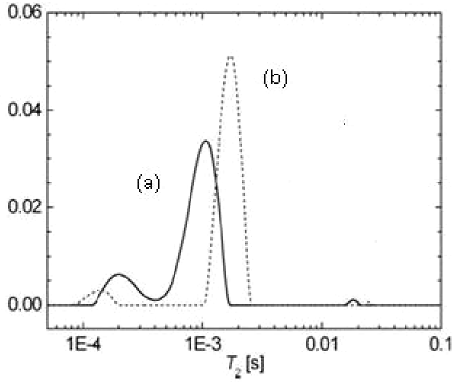

Fig. 3. *The spin-spin relaxation times T_2 distribution of starch (a) and 85% starch + 15% water (b)*

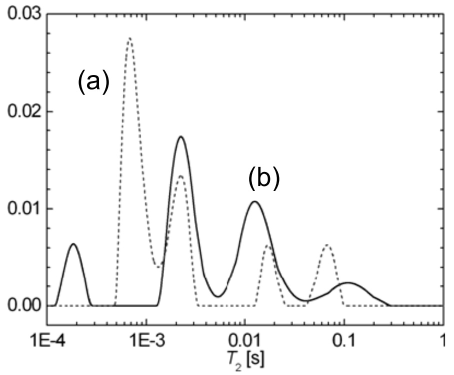

Fig. 4. *The spin-spin relaxation times T_2 distribution of 80% starch + 20% glycerol (a) and 68% starch +17% glycerol +15 % water (b)*

The peak from T_2 ~ 0.2 ms may be associated with starch polymer chain segments with the lowest mobility (A – type structure) due to the junction of one end of amylopectin branches to backbone chain. The second component with T_2 ~ 1ms may be associated with more mobile amylose and/or amylopectin polymer chain segments located away from junctions in core branches region (B – type centers). The minor T_2 component located at ~ 20 ms presents a dynamic liquid-like behavior and can be associated with the mobile lateral branches of the amylopectin microstructure or free chains of amylose microstructure (amorphous). The small integral area of this peak suggests that the percentage of lateral branches is small, leading to the idea that the starch molecules are closely packed (crystalline).

By adding 15 % of water one can observe a displacement of semi-mobile (T_2 ~ 1ms) and mobile (T_2 ~ 20ms) peaks to higher T_2 values and a decrease of the rigid component (T_2 ~ 0.2 ms). Thus the additional water content lead to a larger mobility of amylopectin polymer chain segments in the core packed branches.

The mixture between starch with glycerol (Fig.4a) leads to a distribution characterized by four peaks. The significant dynamic components remain in the semi-mobile region (peaks centered at T_2 ~0.6 ms and 2.5 ms) and the other two peaks situated at larger T_2 values of ~20 ms and ~70 ms suggest that glycerol increases the number of lateral branches and may create mobile free ends in amylose and amylopectin polymer chain segments finally leading to an increase of mobility of the starch polymer chains (amorphous).

An increase in intensity of the last three major components can be observed in the T_2 distribution for the mixture of starch with glycerol and water (Fig.4b). This can be interpreted as a transformation of amylopectin microstructure where the lateral branches are moved away from the core branches with the creation of more mobile free ends (amorphous). However, it should be noted that the presence of additional water molecules can maintain a rigid dynamic component (crystalline) of the starch polymer chain segments (peak located at ~ 0.2 ms) corresponding to amylopectin segments around junctions.

The ^{13}C CP/MAS NMR spectrum (figure 5a) suggest the presence of both crystalline and amorphous phases in the case of the 68% starch/ 17%glycerol/ 15%water sample as shown also by the NMR relaxation data (figure 4b).

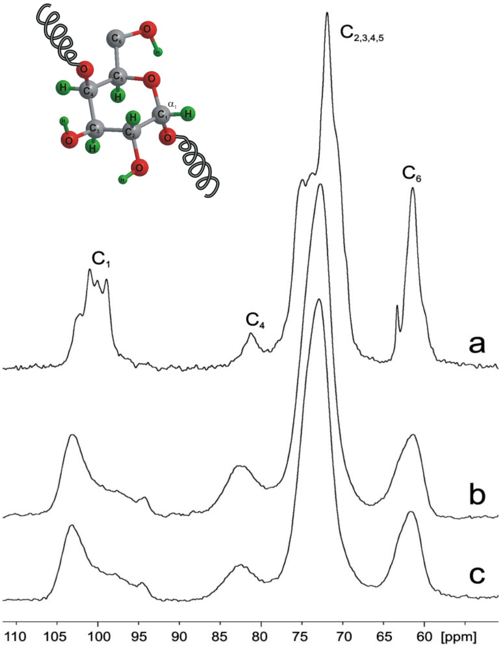

Fig. 5. *The ^{13}C CP/MAS NMR spectra of starch / glycerol / water [mass %] formula with 68/17/15 ratio, a – before and b – after extrusion and c – commercial USA extruded product, recorded at room temperature and 14 kHz spinning speed*

Before extrusion, the A, B and V- crystalline structures are present (figure 5a). In the region of the glucose unit C1 NMR resonance there is an overlap of the *triplet* at $\delta = 101$ ppm (A phase), the *doublet* at $\delta = 101$ ppm (B structure) and a *singlet* at $\delta = 104$ ppm (V type structure).

The strong C 2,3,4,5 *quadruplet* from δ = 73 ppm is characteristic of A – type phase.

The intense C6 absorbtion (δ = 62 ppm) is due to contributions from A, B, and V – types crystalline phases (Wang et al., 2009; Therien and Zhu, 2009). All these absorbtions are situated over the weak and broad NMR resonances at δ = 104 ppm (C1), δ = 83 ppm (C4) and δ = 61 ppm (C6) characteristic of the amorphous phase as in the extruded samples (b, c).

The sharp ^{13}C line at δ = 64 ppm belongs to inclusion glycerol – amylose complex stabilized by hydrogen bonds.

Degradation process

Environmental degradation of biopolymers may be defined as the scission of chemical bonds in the polymer backbone by the attack of water to form oligomers and finally monomers (Shah et al., 2008). The degradation of starch – based packaging required unstable and hydrolysable linkages where chemical, biological or photochemical reactions can take place with the disintegration into their monomers (Wollerdorfer and Bader, 1998).

By following the absorbed water process during five days we found that the product with the high starch content (78/19.5/2.5 starch/glycerol/water – (a)) is most resistant and start to be degradated after only five days (figure 6a).

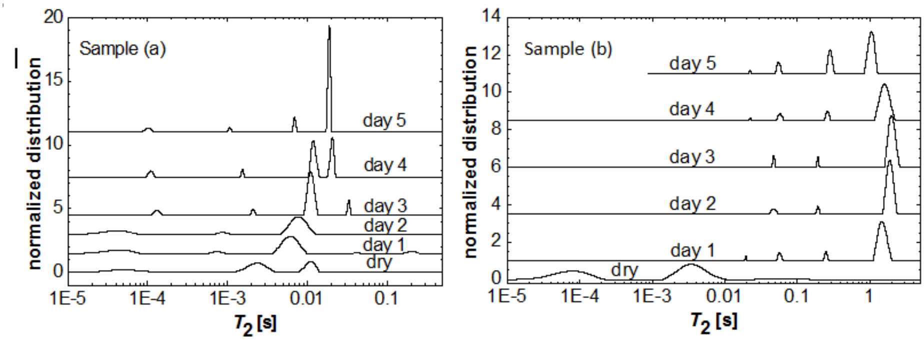

Fig. 6. The normalized T_2 relaxation times distributions of packages samples (a) and (b) for 5 days water absorption compared with dry sample

A semi-mobile phase (T_2 ~ 2.5 ms and ~11 ms) and also a rigid region (T_2 ~ 50 μs) due to the junctions of lateral branches with the amylopectin polymer backbone can be observed for dry sample (a). After one day the two mobile peaks collapses to a unique peak located at T_2 ~ 6.3 ms (figure 6).

Dramatically changes can be observed starting with day 3 when that the T_2 peaks becomes more narrow indicating a reduced distribution compared dray sample or with not so degraded sample (first two days). Their shifts to larger T_2 values compared with the values measured in day 2, suggest the presence of most mobile components.

The product with lower starch content (68/17/15 starch/glycerol/water – (b)) is degraded after one day becoming a colloidal aspect. The dry sample (b) is characterized by two relative rigid components ($T_2 \leq 5$ ms). The amorphous phases prevail in this sample ($T_2 > 10$ ms) after one day (figure 6).

With day 4 a precipitation validated by the decrease in intensity and shifted at lower values of the main peak (more mobile $T_2 \sim 1$s) can be observed for sample (b). A similar behavior was also observed for commercial USA specimen.

CONCLUSION

The IR and Raman methods not give a clear response on the amorphous or crystalline content in starch samples because the vibrational bands have the origin in the same atomic groups of the major starch components.

The relaxation NMR spectroscopy shows that the amorphous / crystalline ratio depends on amylose / amylopectin mobility and also by the place of their polymer chain segments. The distributions of spin – spin (T2) and spin – latice (T1) relaxation times values and theirs shift toward higher values show that the increasing of water and glycerol content in the starch package samples (68/17/15) lead to the more mobile amylose and amylopectin polymer chain segments and the prevalence of amorphous phase.

^{13}C CP/MAS NMR spectra show also that in the extruded samples the amorphous phase becomes dominant.

The most amorphous package with starch/glycerol/water of 68/17/15 mass% ratio is extremely degraded after just one day by the water absorption, forming a colloid substance. The package with a large content of starch (78/19.5/2.5) is most resistant, starting to be decomposed after five days.

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