

INTRODUCING LETTER

The submitted paper describes the results of a part of my PhD work carried out at the Department of Innovation Engineering of University of Lecce (Italy), under the supervision of Prof. Alfonso Maffezzoli, and for six months at the Division of Food Sciences of University of Nottingham, UK, under the supervision of Prof. John R. Mitchell and Dr. Imad Farhat, thanks to a Marie Curie Fellowship financed by the European Community (EC Contact Number: HPMT-CT-2001-00404).

The research performed during the stay in UK, has concerned the “food polymer science”, a relatively new science field in which food systems are studied and interpreted by means of experimental theories developed by synthetic polymer science. This approach can unify structural aspects of foods, viewed as kinetically metastable, completely amorphous or partially crystalline polymer systems with functional aspects dependent on mobility and interaction with solvent, e.g. water.

The results of the retrogradation studies from different techniques (X-ray diffraction, differential scanning calorimetry, dynamic mechanical analysis) have been compared with the ultrasonic results obtained using an ultrasonic wave propagation apparatus developed at the University of Lecce.

A MULTI-TECHNIQUE APPROACH TO THE MONITORING OF THE WHEAT STARCH RECRYSTALLIZATION DURING AGEING

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Introduction

This paper presents the results of a study on the retrogradation of wheat starch extrudates carried out through the combined use of several experimental techniques. The results of the ultrasonic monitoring of starch extrudates recrystallisation during ageing in the rubbery state are compared with the results of other experimental techniques already used to study the retrogradation of starch based biopolymers, such as X-ray diffraction, differential scanning calorimetry and dynamic mechanical analysis

The retrogradation kinetics of wheat starch samples is modelled by the Avrami equation and the potential of ultrasonic technique in monitoring the retrogradation process of concentrated wheat starch systems is proved.

Keywords: DSC, XRD, ultrasonic, dynamic mechanical analysis, wheat starch, retrogradation, ageing.

Problem statement and research goal

Starch is the main component of many human and animal diets. It is found in the storage organs of plants in the form of partially crystalline water-insoluble granules, the size and composition of which depends on the botanical source. Starch consists mainly of two polysaccharides, amylose (linear polymer) and amylopectin (highly branched macromolecule). Amylopectin is believed to be the main contributor to the crystallinity of the native starch granule [1].

The starch granules are made of concentric amorphous and semi-crystalline growth rings. These latter are composed of alternative crystalline and amorphous lamellae [2]. The crystalline lamellae are made of double helices of amylopectin, which are packed according to either a monoclinic unit cell, this is known as the A polymorph, or a hexagonal unit cell, this is known as the B polymorph. The A-type crystals contain 4 water molecules while the B-type hold 36 water molecules per 12 α -D-glucopyranosyl monomers [1, 2,3].

Starch granules are insoluble in cold water, but, when heated in the presence of excess water, they swell. This swelling is reversible up to a certain temperature, known as the gelatinisation temperature, where a more pronounced swelling, accompanying the melting of the crystalline regions, occurs and the principally linear amylose becomes soluble and leaches out of the disrupted granule. In addition to thermal processing, the conversion of starch from its native partially crystalline granular structure to a polymeric solution/melt and subsequent molecular degradation can be driven by mechanical shear (e.g. in extrusion), chemical (e.g. through the use of solvents) and biochemical (e.g. through enzymatic hydrolysis) processes, etc.

On cooling and during early storage of converted/gelatinised starch, amylose gelation or retrogradation occurs [4] while, on longer time scale storage (hours-weeks depending on composition and storage conditions), amylopectin retrogradation occurs, which essentially leads to the partial recrystallisation of amylopectin producing an increase of firmness and a decrease of water-holding capacity [3]. Retrogradation happens because gelatinised starch is often supercooled and stored below its melting temperature and therefore is not in thermodynamic equilibrium and, during storage, starch molecule packing and crystallisation occurs. Starch retrogradation is scientifically and technologically important since it leads to significant changes in the mechanical properties of starch-based products and thus greatly affects their sensory (e.g. texture and flavour perception), nutritional (susceptibility to amylosis [5]) and processing (shredding, cutting, etc.) characteristics. Consequently, numerous investigations have been carried out on this phenomenon [1-5, 6-7] but the molecular level mechanisms involved are not fully understood. Furthermore, a main body of existing knowledge relates to dilute starch systems, which are of limited relevance for many food systems.

The aim of this research was to use a range of techniques to study the changes occurring during storage of concentrated wheat starch systems. This study is part of major research programme aimed at gaining an enhanced molecular understanding of transformations occurring during the processing and storage of starch systems (starches from different botanical sources and a range of starch containing foods) by using a range of complementary techniques which probe various physical properties over different distances and timescales, applied on the same materials, subjected to the same processing and storage condition, as often misleading comparisons are done between techniques performed in different studies and conditions.

Experimental techniques

Sample preparation

Non expanded wheat starch/water ribbons were prepared by extrusion through a 1 x 30 mm slit die using a Cleextral BC-21 co-rotating intermeshing twin extruder. The extrusion temperature profile was 40, 90, 120, 75 °C, the feed rate of solids was 5 kg/h and the screw speed was 300 rpm. Different amounts of distilled water were introduced into the second zone of the extruder barrel to obtain samples containing 37% or 51% water on a dry weight basis (w/w d.s.b.) i.e. grams of water per 100 grams of dry solid. Water contents were determined directly after extrusion by drying the sample at 105 °C for 24h.

The samples were sealed in airtight aluminium bags (for the XRD and DMA studies) or in stainless steel DSC pans (for the calorimetry study) to prevent loss of water and stored in an incubator at 25 ± 1 °C.

X-ray diffraction

Wide angle X-ray diffraction (XRD) measurements were carried out to obtain information on long-range crystalline order. A Bruker AXS D5005 diffractometer was used. The X-ray generator was equipped with a copper tube operating at 40 kV and 30 mA and irradiating the sample with a monochromatic Cu K_α radiation with a wavelength of ~0.154 nm. XRD spectra were acquired at room temperature over the 2θ range of 4-38° at 0.1° intervals with a measurement time of 6s per 2θ interval. The angular range encompassed all the diffraction peaks of starch crystals.

XRD diffractograms were acquired at regular storage time intervals up to 10 days of storage on disks (~25 mm of diameter) cut from the extruded ribbons.

Differential scanning calorimetry

The samples were sealed in high-pressure stainless steel pans and stored at 25°C as described above for different times prior to analysis. The melting behaviour of the retrograded starch was studied using a power compensated Perkin-Elmer DSC 7 differential scanning calorimeter. An empty stainless steel pan was used as a reference. The samples were heated from 20°C to 160 °C at 10°C/min. A second heating scan after a rapid cooling of the samples did not show any thermal event in the amylopectin-melting region. The thermograms were normalized to the dry matter weight of each sample. The sample contained in the stainless steel pan was weighed before and after heat treatment to check for potential moisture loss.

Dynamic Mechanical Analysis

Dynamical mechanical analysis (DMA) was used to monitor the changes in the mechanical properties and the macromolecular relaxation processes occurring during retrogradation.

A Polymer Laboratories dynamic mechanical analyser operating in bending mode was used. Rectangular strips (approximately 7 mm x 14 mm x 2 mm) were cut from the extruded ribbons and clamped in the single cantilever geometry. The analysis was performed during heating from -30 to 70°C at six frequencies between 0.3 to 30 Hz. A relatively low heating rate of 1°C/min was used to insure adequate thermal equilibrium across the sample. In order to alleviate potential water loss during the measurement, the sample was covered with a thin film of silicon oil and no data were acquired above 70°C.

The results of dynamic mechanical analysis are valid only if the material exhibits linear viscoelasticity, which implies that E', E'' and tan δ must be independent on the strain amplitude. Therefore, particular attention was devoted to the choice of the strain amplitude. A preliminary test at fixed frequency and variable strain (strain sweep test) was performed to determine the strain amplitude values that did not affect the moduli values and, based on these results, a strain amplitude of 0.1% was selected.

Ultrasonic analysis

Ultrasonic longitudinal velocity and attenuation during ageing at 25°C of concentrated wheat starch systems were measured by transmission mode at the frequency of 10 MHz by means of an ultrasonic apparatus developed in the laboratory of Polymeric Materials of Lecce University. It consists of two specifically developed ultrasonic transducers, fitted into the disposable tools of a parallel plate rheometer (Ares, Scientific Rheometric) and connected with a pulser-receiver card. This latter generates a pulse train, amplifies the signal transmitted through the sample and provides an analogue/digital conversion of the signal, that, using a dedicated software, is sampled every 5s and displayed on the monitor of a PC.

The contact pressure and the sample thickness are controlled by the vertical movement of the upper transducer, driven by the gap control function of the rheometer, while the temperature is controlled by the air forced environmental controller of the rheometer oven.

The starch sample, in a form of a disc (12 mm diameter and 1.8 mm thickness) was sandwiched between two ultrasonic probes fitted into the oven of a parallel plate rheometer (ARES, Rheometric Scientific). To prevent water loss during 10 days lasting measurements, the measurement system was sealed with silicone.

The samples were weighed before and after the ultrasonic measurements, but not significant moisture loss was observed. The ultrasonic set-up enabled to monitor continuously the changes in the acoustical properties as a function of the time.

Results and discussion

X-ray diffraction and calorimetric measurements on aged wheat starch

The XRD spectra of wheat starch samples, containing 51% water (w/w d.s.b.), stored at 25⁰ C up to ten days are shown in Figure 1. The native wheat starch granules have an A-type crystalline structure. This long-range order is largely disrupted by the extrusion process, as suggested by the absence of any significant peak related to A-type crystallites in the diffractograms acquired shortly after extrusion, which show only a sharp peak centred at around $2\theta = 19.7^{\circ}$. This peak do not change significantly in intensity nor width with the storage time and can be assigned to the presence of crystalline V-type amylose-lipid complex, which are likely to have formed during the extrusion process [8].

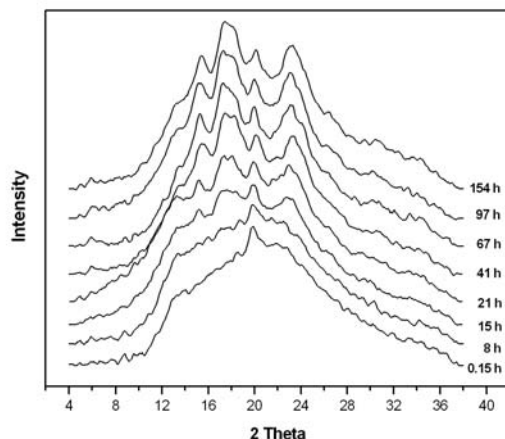


Figure 1 XRD spectra of wheat starch extrudates containing 51% water (w/w d.s.b.) stored at 25⁰C for different ageing times.

On storage, well-defined diffraction peaks emerge indicating an increasing fraction of starch in the crystalline form due to amylopectin retrogradation. Significant variations in the XRD spectra are observed starting from 15 hours of ageing for samples with higher water content. The narrowing of the peaks and the shift to large angles, as the storage time increases, is compatible with an increased size of the crystalline regions and closer molecular packing. After approximately 2 or 3 days of ageing, depending on the moisture content (51% or 37% water d.s.b., respectively), only slight changes in the XRD spectra are observed indicating that the retrogradation process is nearly completed. The polymorph, formed as a result of the recrystallization of the gelatinised starch (mainly amylopectin in these conditions) during ageing, depends largely on the water content and storage temperature and is not necessarily the same as that of the original native starch [8, 9, 10, 11].

The DSC thermograms of retrograded wheat starch sample with 51% water content are shown in Figure 2.

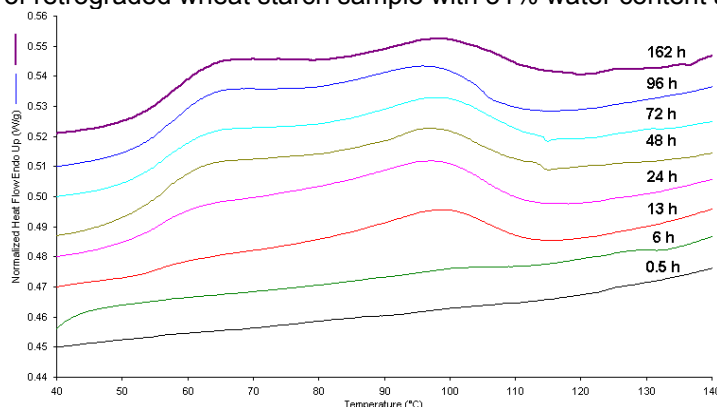


Figure 2 Melting endotherms of wheat starch extrudates (51% water w/w d.s.b.) stored at 25⁰C for different times and heated from 20 to 160⁰C at 10⁰C/min.

The thermograms acquired shortly after extrusion show no significant thermal events in the region between 25°C and 140°C except for a minor endotherm centred at ~127°C which is likely to be related to the melting of the complex between amylose and the endogenous starch lipids, as seen in the XRD data. As the storage time increases, a bimodal endothermic event with components centred, for the sample containing 51% water

at ~100°C and ~63°C, become increasingly significant, i.e. an increasing melting enthalpy is observed. These endotherms are assigned to the complex melting behaviour of starch crystallites in limited water content conditions.

The temperature range over which melting occurs is very broad (from ~50 to ~115 °C) suggesting a wide range of “melting” events. These could be attributed to a broad distribution of crystals with varying stability. Amylopectin recrystallization or crystal perfection phenomena could take place during the DSC heating scan owing to an increased molecular mobility. In this case, chain rearrangements just above the onset of melting [7,12] could lead to a larger equilibrium with melting at higher temperatures. Another more likely interpretation for this melting pattern is that the low temperature endotherm is related to the disruption of the packing of double-helices of the amylopectin A-chains while the high temperature endotherm reflects the subsequent dissociation of these double helices.

In order to assess the recrystallisation kinetics, the degree of crystallinity has been calculated using the Wakelin correlation method [13]. According to this method, the crystallinity index χ of a sample is calculated relatively to a minimum and a maximum value corresponding to an amorphous ($\chi=0$) and a crystalline ($\chi=1$) reference standard respectively.

For each scattering angle 2θ in the investigated range, the intensity of the amorphous standard (I_a) at 2θ has been subtracted from that of the sample of unknown crystallinity (I) and of the crystalline standard (I_c) at the same scattering angle. The intensity difference ($I-I_a$) between the sample and the amorphous standard has been then plotted against the intensity difference (I_c-I_a) between the crystalline and amorphous standards. The slope of the resulting regression line provides an estimate of the relative crystallinity index, given by:

$$(I - I_a) = \chi (I_c - I_a) \quad \text{Eq.1}$$

In this work the patterns of a sample analysed shortly after the extrusion and a fully retrograded sample have been taken as the amorphous and crystalline standards, respectively. The correlation method is easy to automate and adopt for routine analysis but suffers from the assumption that the fresh sample is totally amorphous and the “fully” retrograded sample is maximally crystalline.

The melting enthalpy of the retrograded amylopectin has been calculated from the area of the combined endotherm as an indication of the extent of retrogradation and has been expressed on the basis of the dry matter weight of each starch sample. In Figure 3 the normalized DSC melting enthalpies are compared with the XRD crystallinity indices calculated according to the Wakelin correlation method. The progress of retrogradation monitored by the two different techniques is comparable with the sample of lower moisture content (37% w/w d.s.b.) showing a slower retrogradation rate than that with 51% water (w/w d.s.b.). Nevertheless, it is possible to suggest that in the initial stages, at least for the 37% water content, the extent of order detected by DSC exceeds that measured by XRD. This is compatible with the interpretation proposed above for the bimodal melting behaviour.

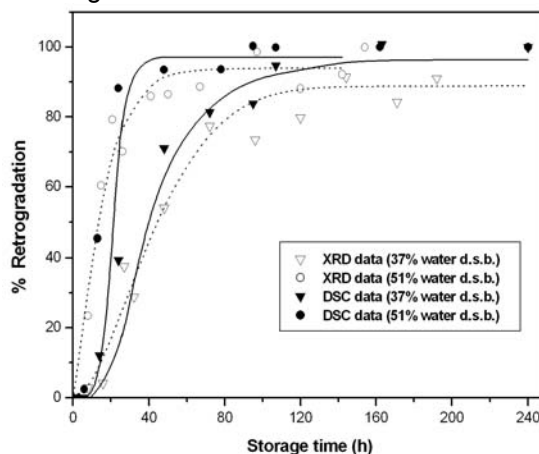


Figure 3 Comparison of the extent of retrogradation calculated from XRD data (through the crystallinity index) and from DSC data (through melting enthalpy) on wheat starch extrudates with two different moisture contents (37% and 51% w/w/ d.s.b.) and stored at 25°C. The dotted lines are the Avrami fits to the experimental results.

Since the DSC and XRD results are essentially comparable, the choice of one or the other of these techniques will depend on practical requirements. The advantages of DSC are that no water loss occurs over the course of storage due to the perfect sealing of the sample inside the DSC pans and a direct measurement of retrogradation through the melting enthalpy and the melting temperature range. XRD provides more conclusive evidence of crystallinity and polymorphism.

Low frequency dynamic mechanical measurements on aged wheat starch

DMA has been used to study the changes in the mechanical spectrum of the extruded wheat starch materials throughout the retrogradation process. Like any semi-crystalline polymer, the amorphous regions of starch undergo molecular relaxation motions, which are constrained by the presence of the crystalline phase. During starch retrogradation, the relative amount of amorphous and crystalline phases changes as a result of recrystallization. As a consequence, the glass rubber transition would be expected to change with ageing time in terms of magnitude (as this transition reports on the amorphous fraction of the material) and possibly its temperature (as usually the glass transition temperature T_g increases with crystallinity). The changes in the macromolecular mobility occurring at the glass transition are therefore an indirect measure of the retrogradation process and can be accurately followed by DMA. This mechanical spectroscopy technique detects the amount of energy absorbed by a material particularly when the measurement frequency approaches that of a given molecular relaxation process. In this case, the retrogradation has been analysed through the behaviour of the amorphous phase of starch rather than that of the crystalline phase, as was the case for XRD and DSC.

The DMTA thermograms at 1 Hz on wheat starch extrudates (51% water d.s.b.) aged at 25°C for different storage times and heated at 1°C/min are shown in Figure 4. Data have been acquired over a range of frequencies from 0.3 to 30 Hz, but for clarity, only the results at 1 Hz are shown. The main loss $\tan\delta$ peak is assigned to the glass transition phenomenon. This has been confirmed by the multi-frequency measurements, where it is clear that the loss peak shifts to higher temperatures as the measurement frequency increases (Figure 5).

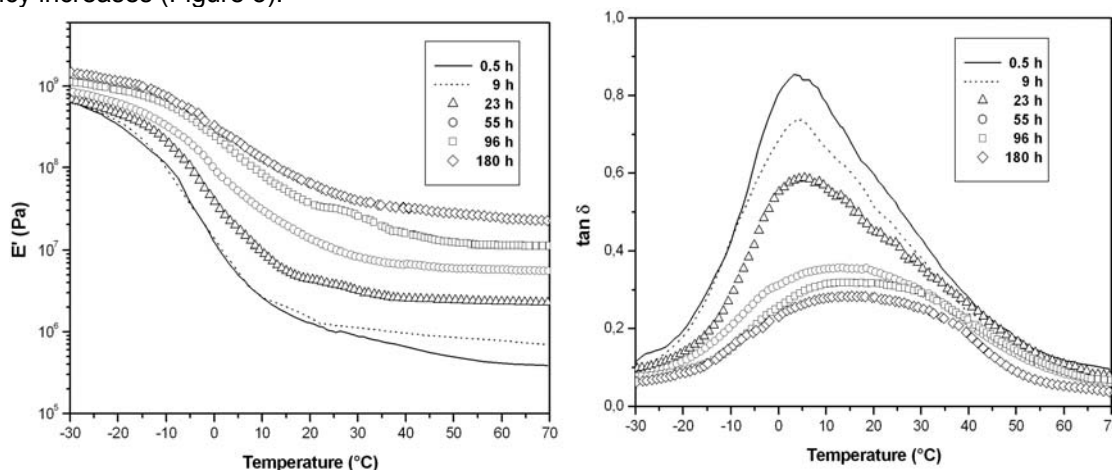


Figure 4 DMA thermograms at 1 Hz acquired on wheat starch extrudates (37% water d.s.b.) stored at different times at 25°C and heated at 1°C/min.

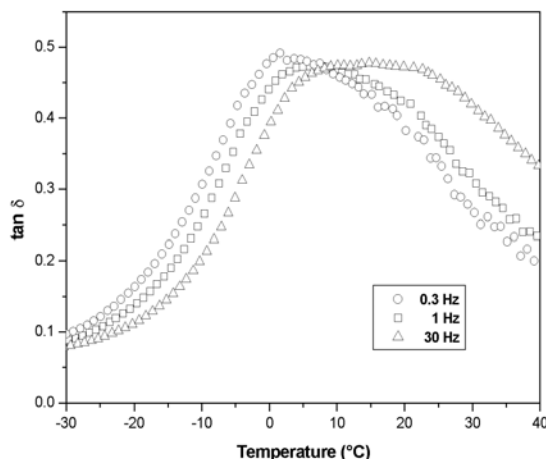


Figure 5 Multi-frequency comparison of $\tan\delta$ peaks for wheat starch extrudates (37% water d.s.b.) stored at 25°C for 24 hours and heated at 1°C/min.

The initial unrelaxed value of the storage modulus E' (at -30°C) is similar for all the investigated times, while the relaxed value (in the rubbery state) depends strongly on storage time and consequently, of the degree of crystallinity of the sample (Figure 4). This increase of the relaxed modulus with the time reflects the increase of the crystallinity fraction in the material that reduces the amorphous phase and, consequently, the entity of the glass transition. This can be observed not only from the reduction of the storage modulus but also from the decrease of the $\tan\delta$ peak with the time. The initial value of the loss modulus E'' increases in the first part

of the crystallization process as the crystallinity increases. Although the relaxation originating the loss is assumed to occur mainly in the amorphous regions, the increase in the energy dissipation may be related to the interaction between amorphous and crystalline regions.

The consequence of such interaction is also the increase of the width of $\tan\delta$ peak with increasing storage time, i.e. with increasing crystalline fraction in the sample. The presence of a more significant crystalline phase could hamper the relaxational motions of the amorphous phase, leading to a decrease in the height of $\tan\delta$ peak. In summary, the reduction of the entity of transition with ageing could be attributed to the concurrent decrease both of the amorphous regions and their mobility in the starch sample and to a reorganization of water molecules inside the sample with a consequent increase in the amount of bound water in starch structure.

The storage modulus measured by dynamic mechanical analysis, can be an useful measurement of the progress of the retrogradation, being its increase related to the growth of the crystalline phase and to the strong interactions between amorphous and crystalline regions inside the material. The development of the mechanical properties of wheat starch extrudates stored at 25°C for different times and tested at 1 Hz is shown in Figure 6.

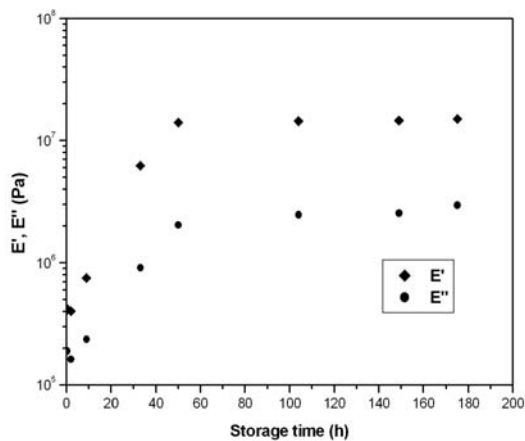


Figure 6 Development of the mechanical properties of wheat starch extrudates (51% water d.s.b.) tested at 25°C and 1 Hz after ageing at different times.

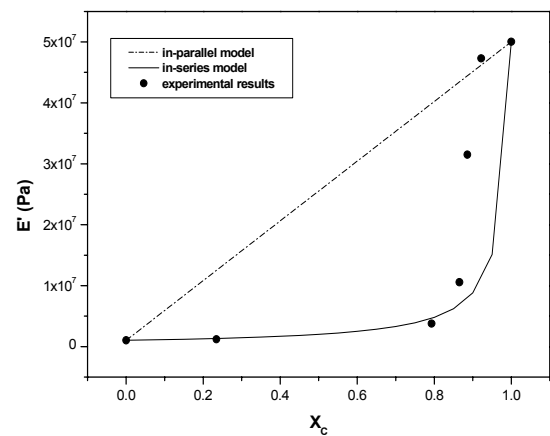


Figure 7 Storage modulus measured at 25°C and 1Hz for wheat starch extrudates (51% water d.s.b.) plotted as a function of the degree of crystallinity: comparison among in-parallel model, in-series model and experimental results.

Aged wheat starch samples can be viewed as composite materials, in which the crystalline phase is like a rigid inclusion in the amorphous matrix (rubber-like phase). Considering such a biphasic system, the development of starch storage modulus as a function of the crystallinity content, during ageing at 25°C, can be described by different mathematical models used in the micro-mechanical theory of composite materials [14, 15, 16].

The upper and lower bounds for the dependence of the composite modulus E^* on the volume fraction X_c of the crystalline component for a two-phase model are given by an "in-parallel" and "in-series" model of semicrystalline and amorphous phases. Assuming that both crystalline and amorphous phases are subjected to the same strain, the in-parallel model can be applied using the well-known mixture rule [15]:

$$E^* = (1-X_c)E_a^* + X_cE_c^* \quad \text{Eq. 2}$$

where E^* , E_a^* and E_c^* are the complex moduli of the sample, of the amorphous and crystalline phases, respectively, and X_c is the degree of crystallinity calculated from XRD experiments. The real and imaginary components of equation 3 can be easily separated giving the following expression of the storage modulus:

$$E' = (1-X_c)E'_a + X_cE'_c \quad \text{Eq. 3}$$

On the other hand, assuming an in-series connection of amorphous and crystalline phases, in which they are subjected to the same stress, the complex modulus can be modelled according to the following equation:

$$\frac{1}{E^*} = \frac{X_c}{E_c^*} + \frac{(1-X_c)}{E_a^*} \quad \text{Eq. 4}$$

from which the storage modulus can be calculated as [15]:

$$E' = \frac{E'_c X_c (E'_a{}^2 + E''_a{}^2) + E'_a (1-X_c) (E'_c{}^2 + E''_c{}^2)}{[E'_c (1-X_c) + E'_a X_c]^2 + [E''_c (1-X_c) + E''_a X_c]^2} \quad \text{Eq. 5}$$

The change of the storage modulus, measured at the frequency of 1 Hz, as a function of the degree of crystallinity, is reported in Figure 7. The experimental results showed a behaviour very close to the in-series

model up to a degree of crystallinity of 0.8. At higher crystallinity fractions, there is probably a phase inversion with the crystalline phase becoming the continuous phase. In such case, both crystalline and amorphous phases would be subjected to the same strain, explaining the similarity with the in-parallel model.

Ultrasonic monitoring of wheat starch retrogradation

Ultrasonic wave propagation has been used to follow the reordering process of starch extrudates during ageing. Since starch retrogradation is accompanied by a recrystallisation process, the changes in sample stiffness, due to the growth of crystalline fraction inside the sample, affect the viscoelastic properties of the material, which can be measured by the ultrasonic technique.

The evolution of the ultrasonic velocity with ageing time is shown in Figure 8 for starch extrudates containing 51% water (w/w d.s.b.). The overall increase in the ultrasonic velocity observed in 10 days is about 100 m/s and can be attributed to the weak development of elastic properties in the retrograding starch system due to the presence in the sample of a high amount of water with, consequently, a strong plasticizing effect. It should be kept in mind that the ultrasonic measurements have been carried out on samples in the rubbery state, being the test temperature much higher than the glass transition temperature.

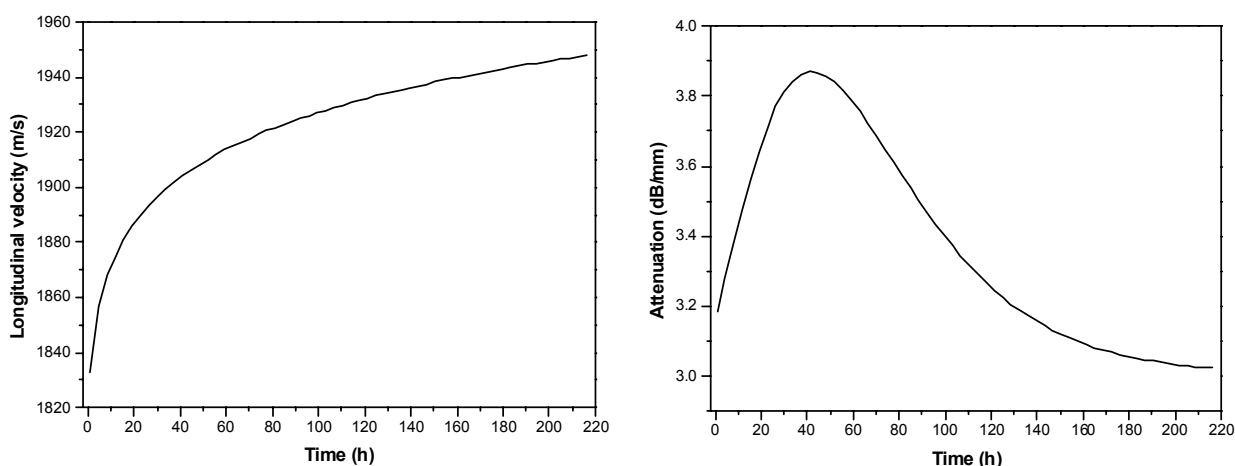


Figure 8 Evolution of the ultrasonic velocity (left) and attenuation (right) (measured at 10 MHz) versus ageing time for wheat starch sample with 51% water (d.s.b.). The solid line is the fit of the experimental results.

The absence of water loss in the samples indicates that the velocity increase in the development of elastic properties is due to the increase of crystalline fraction in starch samples. After about 24 hours of ageing, the ultrasonic velocity continues to increase with smaller rate for very long time. After ten days of measurement, the ultrasonic velocity has not yet reached a plateau value, which, on the contrary, has been observed in the crystalline index and melting enthalpy. This means that ultrasonic technique is sensitive to detect very small change in the viscoelastic properties of starch samples during retrogradation.

The evolution of ultrasonic attenuation versus ageing time for wheat starch extrudates containing 51% water (w/w d.s.b.) is shown in Figure 8. The wave attenuation presents an initial sudden growth followed by a maximum and finally a slow levelling off at a lower value than the initial one. The decrease of attenuation between initial and final values may be attributed to the change of mechanical properties of the retrograding system.

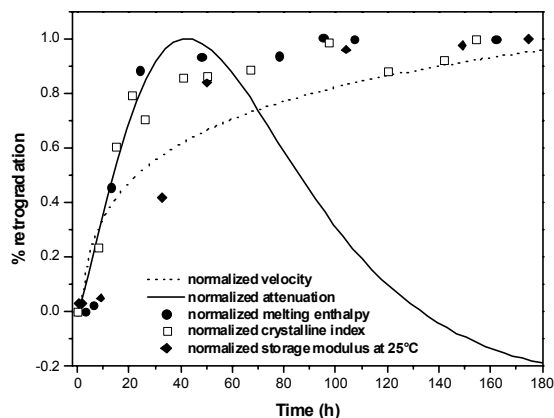


Figure 9 Comparison of the relative extent of retrogradation at 25°C of wheat starch extrudates (51% water d.s.b.) as sensed by different techniques.

The ultrasonic attenuation for starch sample with 51% water (w/w d.s.b.) presents a peak after about 35 hours of ageing, i.e. nearly in correspondence of the plateau value reached by the crystalline index calculated from XRD data and by melting enthalpy measured by DSC (Figure 9). On further ageing, a strong decrease in the attenuation value and a small increase in the velocity curve is observed from ultrasonic experiments, while XRD and DSC measurements indicate no more growth of crystalline fraction inside the sample. Therefore, the behaviour of ultrasonic data can be explained hypothesizing that, after the maximum development of crystalline fraction, a reorganization of the crystallites occurs. The consequent more homogeneous distribution of the crystallites leads to a slight stiffening of the starch sample and to the reduction of the ultrasonic absorption.

Since the sample dimension in the normal direction to the propagation direction of the acoustic wave is large compared to the wavelength, the wave propagation is governed by the complex bulk longitudinal modulus L^*

$$L^* = L' + iL'' \quad \text{Eq.6}$$

whose real and imaginary components can be calculated as [17]:

$$L' = \frac{\rho c^2 \left[1 - \left(\frac{\alpha \lambda}{2\pi} \right)^2 \right]}{\left[1 + \left(\frac{\alpha \lambda}{2\pi} \right)^2 \right]^2} \quad \text{and} \quad L'' = \frac{\rho c^2 \left(\frac{\alpha \lambda}{2\pi} \right)}{\left[1 + \left(\frac{\alpha \lambda}{2\pi} \right)^2 \right]^2} \quad \text{Eq. 7}$$

For the studied case, the term $\alpha\lambda/2\pi$ remains always lower than 0.05, so the contribution of attenuation can be neglected. Therefore, considering the system as a low damping structure, L' and L'' have been calculated as:

$$L' = \rho c^2 \quad L'' = 2\rho \frac{c^3 \alpha}{\omega} \quad \text{Eq.8}$$

where c is the ultrasonic velocity, α the attenuation, ω the angular frequency and ρ the density of the wheat starch extrudates (1323 kg/m^3).

The time dependence of L' and L'' , reported in Figure 10, is dominated by the velocity and follows the same pattern of Figure 8.

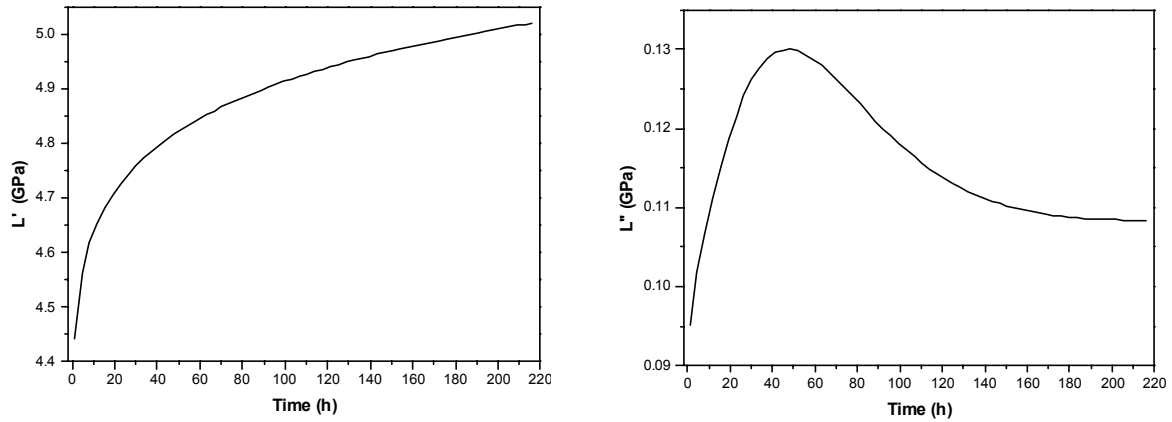


Figure 10 Evolution of the storage and loss component of the longitudinal modulus versus ageing time for wheat starch sample with 51% water (d.s.b.).

Modelling the retrogradation kinetics using the Avrami model

The results obtained from the different techniques used in this study have been fitted to the Avrami kinetics equation [18] to model the retrogradation kinetics of starch samples [19]. During retrogradation, the starch molecules recrystallise in a gradual way and the amount of amorphous material at time t , $U(t)$, has an exponential time dependence described by the Avrami equation [18]:

$$U(t) = \exp(-kt^n) \quad \text{Eq. 9}$$

where k is the kinetic constant and n is the Avrami exponent, related to the dimensionality of nucleation and crystal growth. If the changes in X-ray crystallinity indices, retrogradation enthalpies and ultrasonic velocities are assumed to be measures of the extent of crystallization, then the amount of amorphous material $U(t)$ can also be described by the following equation:

$$U(t) = \frac{Y_{\infty} - Y(t)}{Y_{\infty} - Y_0} \quad \text{Eq. 10}$$

where $Y(t)$ is any physical parameter describing the retrogradation process among those above mentioned. Y_0 and Y_{∞} are the experimental values of physical parameters measured at time 0 and ∞ , respectively. The above two equations can then be combined and rearranged to yield the following:

$$Y(t) = Y_{\infty} - (Y_{\infty} - Y_0) \exp(-kt)^n \quad \text{Eq. 11}$$

The experimental data from different techniques are fitted to the Avrami equation. The fitted crystallisation kinetics are shown in Figure 3 and Figure 11.

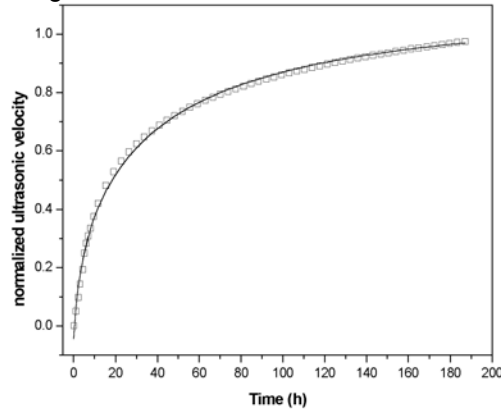


Figure 11 Effect of ageing on normalized ultrasonic velocity for extruded wheat starch with 51% moisture content. The solid line is the Avrami fit to the experimental results.

It should be noted that the different techniques do not give the same kinetics because they monitor different properties and different aspects of the retrogradation process. DSC, ultrasonic wave propagation and DMA monitor alterations in physical properties such as, respectively, the latent heat of melting of crystalline regions, the acoustic properties and the development of a mechanical behaviour due to the increase in rigidity provided by the retrogradation. XRD studied changes in long-range three-dimensional order in crystalline starch domains. Therefore, comparisons of kinetics data from different techniques must be made with caution. From the multi-technique investigation on retrogradation, complementary information rather than a pure similarity of results must be searched.

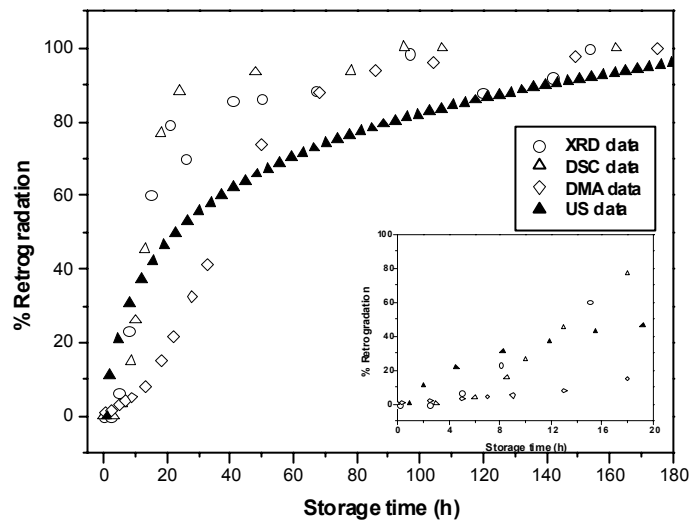


Figure 12 Comparison of the relative extent of retrogradation at 25°C of wheat starch extrudates (51% water w/w d.s.b.) as sensed by different techniques. The extent of the retrogradation in the first 20 hours of ageing is reported in the inset.

Moreover, it is interesting to discuss the sensitivity of each technique to monitor the early stages of the retrogradation process. As shown in Figure 12 for the sample containing 51% water (d.s.b.), significant extent of retrogradation is detected at different times in the early stages of ageing, depending on the technique used. Among the techniques used in this work, ultrasonic wave propagation has the highest sensitivity to monitor the first changes in the material due to retrogradation. This technique is likely to be sensitive to the early stages of the retrogradation process i.e. the short-range re-ordering corresponding to the formation of double helices, which subsequently aggregate to form crystallites. The retrogradation, as detected by DSC and XRD, is observed only two or three hours later, because crystallites can only form once a significant level of short-range reordering has occurred..

The delayed beginning of retrogradation, as detected by DMA through the plotting of the storage modulus at 25°C, can be attributed to the fact that the development of significant modulus would lag behind the development of crystallites detected by both DSC and X-ray diffraction, as a critical volume fraction of the crystalline phase would be required.

Conclusions

In this work, the great potential of the ultrasonic wave propagation as a technique for high frequency dynamic mechanical analysis of polymers has been presented. In particular, the ultrasonic technique has been applied to the monitoring of the retrogradation process of wheat starch extrudates. By comparing ultrasonic results with those other experimental techniques already applied to retrogradation studies, complementary information on the "onset" of the reordering process has been obtained.

Ultrasonic wave propagation is able to monitor starch retrogradation because this phenomenon is accompanied by the recrystallisation of amylopectin molecules and, consequently, by a change in the viscoelastic properties that affect the acoustic behaviour of the material.

The reliability of the ultrasonic results is provided by the absence of water loss during measurements; therefore, the increase in the acoustic properties can be attributed without any doubt to the recrystallisation of amylopectin molecules inside starch sample. Despite off the long time of measurement (about 10 days), the overall increase in the ultrasonic velocity is small (about 100 m/s), due to the high amount of water in starch gels.

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