



Assessing the suitability of polylactic acid flexible films for high pressure pasteurization and sterilization of packaged foodstuff

L. Sansone^a, A. Aldi^b, P. Musto^c, E. Di Maio^a, E. Amendola^b, G. Mensitieri^{a,*}

^a Department of Materials and Production Engineering, University of Naples Federico II, Piazzale Tecchio 80, 80125 Naples, Italy

^b Institute of Biomedical and Composite Materials, National Research Council of Italy, Piazzale Tecchio 80, 80125 Naples, Italy

^c Institute of Chemistry and Technology of Polymers, National Research Council of Italy, Via Campi Flegrei 34, Olivetti Buildings, 80078 Pozzuoli (NA), Italy

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ABSTRACT

Effects of high pressure (HP) pasteurization and sterilization processes on the properties of commercial biodegradable polylactic acid (PLA), used as packaging material, have been investigated. HP treatments have been performed at pressures of 200, 500 and 700 MPa on PLA pouches, containing different types of food, i.e. tap water, solid carrots, carrot juice and carrot puree. Process temperature was in the ranges 25–40 and 90–110 °C, respectively for HP pasteurization and HP sterilization.

To the aim of assessing the suitability of PLA film for HP treatment of packaged food, the effects of treatments have been analyzed in terms of structural/morphological changes (i.e. crystallinity, density, orientation of PLA film) and of thermodynamics and functional properties (i.e. melting and glass transition temperature, permeability and solubility of gases and of water vapor). It has been found that HP pasteurization does not affect to a significant extent any structural and functional property of the treated material relevant for food packaging applications. Conversely, HP sterilization promotes the hydrolysis of the material accompanied by an increase of crystallinity of the film and a decrease of density of the amorphous phase related to the temperature/pressure history. These effects determine an unacceptable embrittlement and opacification of the material that makes it unsuitable for HP sterilization applications.

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1. Introduction

Among novel processing treatments of packaged food, recently introduced to improve safety, quality and shelf-life of foodstuff, high pressure (HP) processing is steadily gaining as a food preservation method that also preserves natural sensory and nutritional attributes of food with minimal quality loss. Packaged foods processed by using this technique maintain most of their original texture and nutritional qualities, additionally exhibiting an extended shelf-life. With reference to preservation of fresh high-quality low-acid vegetables, this technology, as compared with standard thermal processing, better satisfies the consumers preferences for minimally processed food products.

HPP applies high pressure (typically in the range of 300–800 MPa over a time frame of the order of minutes) to packaged foodstuff in order to significantly reduce the number of microorganisms as well as to deactivate enzymes by mechanically-induced mechanisms (Alpas et al., 1999, 2000; Deplace and Mertens, 1992; Farkas and Hoover, 2000; Hayashi, 1989; Neil, 1999). High pressure load is exerted on packaged foodstuff by means of a pressurized confining fluid imprisoned in a vessel. The process consists of

a preliminary heating of both confining fluid and packaged foodstuff, followed by adiabatic pressurization. High pressure pasteurization is performed at an initial temperature of 25 °C while sterilization is conducted at a higher initial temperature of the order of 90 °C. Since the adiabatic pressurization process determines a monotonic increase of the initial temperature, the actual treatment temperature depends on the maximum attained pressure, with temperature raise of 2–4 °C per 100 MPa (Ting et al., 2002). In particular, for the treatment of low-acid food products, HP pasteurization is inadequate in those cases in which also the inactivation of bacterial spores and of some pressure-resistant enzymes is to be obtained. To this aim, HP sterilization is required, since it combines pressure and thermal effects.

Several studies have been carried out on different packaging films demonstrating that packages, in order to be suitable for HP processing, have to present sufficient flexibility to compensate for the collapse of the head space and for the possible volume reduction of the food inside the package (Mertens, 1993; Schauwecker et al., 2002). Moreover, a proper choice of flexible packaging films for HP processing has to be performed in such a way to guarantee that the treatment process does not affect package integrity as well as its functional properties. As a consequence, the selection of packaging material and the design of the package should prevent irreversible deformation phenomena induced by

* Corresponding author. Tel.: +39 081 7682512; fax: +39 081 7682404.

E-mail address: mensitieri@unina.it (G. Mensitieri).

high pressure and severe stress regime (Caner et al., 2000, 2004; Galotto et al., 2008; Morris et al., 2007). In summary, packaging material should insure flexibility while maintaining physical integrity and suitable barrier properties. Moreover, in the case of polymeric films for flexible packaging applications, heat sealing should withstand the mechanical and thermal loading associated with HP process (Khono and Nagakawa, 1990; Kübel et al., 1996).

In fact, HP processing has the potential to produce undesirable effects in some polymeric films, as reported in previous studies showing how some flexible polymer films can lose significant barrier to oxygen, carbon dioxide and water vapor when submitted to these treatments (Caner et al., 2000; Lambert et al., 2000; Schauwecker et al., 2002). In particular, an important issue that should be addressed when considering a polymeric materials for packaging in HP treatments, is the possible effect of treatments on the structure and morphology of the material which, in turn, can determine relevant effects on mechanical, functional (i.e. gas and vapor barrier properties) and esthetic (see-through clarity) properties. In fact, modification of crystallinity level, density change of amorphous regions and possible hydrolysis phenomena could hamper the safe use of the packaging material for these applications.

In this framework, it is of interest to assess the suitability of biodegradable polymers for use as packaging material in HP treatments. Among biodegradable polymers, polylactic acid (PLA) is emerging as a possible alternative to nonbiodegradable glassy polymers as, for example, polyethyleneterephthalate. Although PLA has been known for more than a century, it has only been of commercial interest in recent years, in light of its biodegradability and, in the packaging field, of its suitability for many applications (Auras et al., 2004; Drumright et al., 2000; Ikada and Tsuji, 2000a,b; Lim et al., 2008; Lunt, 1998).

PLA is a biodegradable, thermoplastic, aliphatic polyester derived from renewable resources (i.e. corn starch or sugarcane) by bacterial fermentation used to produce lactic acid (Bogaert and Coszach, 2000; Sawyer, 2003; Tuominen et al., 2002). Lactic acid cannot be directly polymerized to a useful product, because each polymerization reaction generates one molecule of water, the presence of which degrades the forming polymer chain to the point that only very low molecular weights are observed. An alternative synthetic route starts from lactic acid oligomer that is then catalytically dimerized to make the cyclic lactide monomer. Although dimerization also generates water, it can be separated prior to polymerization. Polymerization of a racemic mixture of L- and D-lactides usually leads to the synthesis of poly-DL-lactide (PDLLA) which is amorphous. Use of stereospecific catalysts can lead to heterotactic PLA which is semi-crystalline. Changing the ratio of D to L enantiomers it is possible to control the degree of crystallinity (Auras et al., 2004), thus tailoring many important mechanical and functional properties of interest in packaging applications which are affected by crystallinity level.

Motivated by these premises, the present investigation, which has been performed in the framework of the *NovelQ* European project, is aimed at clarifying the influence of HP processing on the morphological/structural and functional properties of commercial semicrystalline PLA films for use in flexible food packaging applications. Preliminary studies are available in the literature (Ahmed et al., 2009), but analyses have been limited to PLA samples in powder form and there is the need of more detailed studies on HP processing of real PLA packages containing foodstuff. In fact, in the present study suitability of commercial PLA films for HP pasteurization and sterilization has been assessed performing test on PLA pouches containing food submitted to processing conditions which closely mimic those actually employed in industrial applications of HP treatments. A thorough experimental analysis has been performed aimed at determining the effects on PLA films of HP treatments in terms of glass transition and melting temperatures,

overall crystallinity, density of amorphous regions, orientation, mechanical properties and barrier to gases and vapors.

It is noticed here that no experimental comparison has been performed with heat treatment conducted in accordance with standard pasteurization and sterilization processes (which, for the case at hand, that considers as packaged food carrot based products, would be respectively in the order of 85–94 and 116–130 °C) since, in these conditions, the PLA would be certainly brought above its glass transition temperature, thus promoting physical alteration of the material which would be unacceptable for food packaging applications, as we were able to assess by treating at atmospheric pressure the PLA films in air and liquid water at these temperature conditions.

2. Experimental

2.1. Material

The PLA used is a commercial film with tradename *Biophan 121* (Treofan Germany GmbH & Co., Raunheim, Germany), with a thickness of 44 µm. The film is mono-axially oriented in the film processing machine direction (MD) and is made up of three layers; the two external layers are sealable and apparently consists of amorphous PLA, likely made up of D and L lactic acid units in such a relative amount to avoid the crystallization under the usual process conditions used for film production, while the core layer is made of semi crystalline PLA (Busch et al., 2007). The exact proportions of D and L lactic acid units was not specified in the manufacturer's datasheet. In view of the three-layer structure of the PLA film, it has been possible to manufacture pouches with single film packaging, with no need of laminating this film with other materials to impart sealing capability.

2.2. High pressure treatment

Four different types of food, i.e. tap water, solid carrots, carrot puree and carrot juice, were packaged into the pouches to be treated, in an amount of about 60 g per pouch. Carrots (*Daucus carota* var. Yukon) were obtained from local shop in Wageningen and stored at 4 °C for a maximum period of one week prior to use in the HP treatment tests. Before use roots were first washed and hand peeled. The three types of carrot based foodstuff were prepared as follows. (1) Solid carrots disks (8 mm height and 12 mm diameter) were excised from the core of the carrots immediately before packaging in the pouches. (2) Carrot juice was extracted by a juice extractor and vacuum filtered through a muslin cloth. The pH of carrot juice was adjusted to 3.8 by adding citric acid. After vacuum degassing under agitation the juice was stored 4 °C and used for high pressure treatments within 2 h. (3) After cutting into pieces, carrots were steam blanched for 30 min, and the cooled in an ice water bath. After the addition of water, roots were then processed by blending into a puree using a kitchen scale blender. Puree was stored at 4 °C and used within few hours.

Food containing pouches were obtained by sealing the three sides of a folded PLA film sheet with a custom built heat sealing equipment (pouches size equal to 15 × 15 cm²). Heat sealing was performed by hot-bar welding on the three side of a pouch, at a heat-seal bar temperature of 110–120 °C, under an applied pressure of four bars (a closing force of 600 N was applied on a 150 mm × 10 mm surface) exerted for 1–2 s. Before sealing the upper side of the pouches, they were filled with the selected food and air was removed from the head space by vacuum pumping. The filled pouches were then subjected to high pressure treatments, performed in a pilot scale high pressure high temperature unit at Wageningen UR (University and Research center) – Food

& Biobased Research, in Wageningen, The Netherlands. This apparatus was developed by Resato, Solico and Unilever companies in cooperation with Wageningen UR Food & Biobased Research and consists of a single, vertically oriented high pressure vessel of 2.5 L. In a typical experiment, pressure was increased up to the desired value, through volume reduction of pressure transmitting fluid using a plunger at the vessel top (plunger system). Pressure could be built up to 700 MPa in 24 s. The treatment temperature was controlled by using an electric heating jacket and a bottom heater attached to the outer wall of the vessel to heat up the vessel content to a maximal temperature of 90 °C. The pressure transmitting medium used was tap water.

Two types of HP treatments have been performed on pouches, i.e. pasteurization and sterilization. Sterilization was performed by pre-heating the pouch at 90 °C into a water bath for ten minutes, followed by the application of the desired hydrostatic pressure (i.e. 200, 500 and 700 MPa) in the high pressure vessel, for 5 min. Pasteurization was performed with the same procedure but the pouch pre-heating was performed around room temperature (i.e. 28.5 °C). It is worthy of note that, during the process, temperature increases due to the adiabatic heating associated to compression (typically 3–4 °C increase for each 100 MPa pressure increase). As a consequence, in the case of sterilization, temperature reached a maximum value of roughly 115 °C, for the treatment performed at 700 MPa, while for pasteurization, process temperature rose from 28.5 °C to a maximum value of roughly 45 °C, for the treatment performed at 700 MPa. Details on the processes are reported in Table 1.

2.3. Characterization techniques for the evaluation of thermodynamic and structural/morphological properties

Several experimental techniques have been used to determine structural/morphological properties and thermodynamic/functional properties of packaging materials before and after HP treatments.

2.3.1. Differential interference contrast microscopy

The differential interference contrast (DIC) has been performed using an optical microscope BX51 (by Olympus, Hamburg, Germany). Using reflected light in a differential interference contrast configuration, photomicrographs of the surface of transverse section of microtomed films were obtained. By means of the DIC clear specimens which often yield poor images when viewed in bright field illumination, are made clearly visible by optical rather than chemical means. Light is polarized in a single vibration plane by the polarizer before entering the lower modified prism that acts as a beam splitter; next the light passes through the condenser and sample before the image is reconstructed by the objective. Above the objective a second prism acts as a beam-combiner and passes

Table 1
Process history for the industrial HP pasteurization and sterilization treatment at 700 MPa.

Time (min)	Pressure (MPa)	Temperature (°C)
<i>Pasteurization</i>		
0–5	0.1 Constant	28.5
5–10	Ramp from 0.1 to 700	Adiabatic rise from 28.5 to 40
10–15	700	40
15–20	Ramp from 700 to 0.1	Adiabatic decrease from 40 to 28.5
<i>Sterilization</i>		
0–5	0.1	Ramp from 28.5 to 90
5–10	Ramp from 0.1 to 700	Adiabatic rise from 90 to 115
10–15	700	115
15–20	Ramp from 700 to 0.1	Adiabatic decrease from 115 to 90
20–25	0.1	Ramp from 90 to 28.5

the light to the analyzer, where it interferes both constructively and destructively. This technique was used to visualize the multi-layer structure of the films and to evaluate the thickness of external sealable PLA layers.

2.3.2. Confocal Raman microscopy

Raman spectra were obtained using a Confocal Raman Microscope LABRAM Aramis (by Horiba Jobin–Yvon, France). A laser with a wavelength of 532 nm was used as excitation source. The objective used to perform the analysis was a 50× with a long working distance. Profiling along the thickness of microtomed film was performed on untreated and treated PLA to gather information on the polymer structure (i.e. crystalline vs. amorphous domains) along film thickness.

2.3.3. FT-IR spectroscopy

The chain axes of stretched macromolecules in thermoplastic polymer films tend to orient preferentially in the stretching direction (Machine Direction, MD), and the extent of this orientation can be evaluated by measuring the dichroism of suitable IR bands. To gather information on the orientation in the plane of the film, samples were analyzed by transmission FT-IR spectroscopy with polarized radiation (System 2000 FT-IR, Perkin–Elmer, Waltham, MA, USA).

Attenuated total reflectance spectroscopy (FT-IR-ATR) has been used to detect possible changes in the chemical structure of the PLA surface after HP treatments. Spectra were collected with a FT-IR spectrometer (Perkin–Elmer, Mod. Spectrum 100, Waltham, MA, USA) equipped with a single reflection ATR system (Universal ATR accessory with Diamond/ZnSe optics, also from Perkin–Elmer).

2.3.4. Thermally modulated differential scanning calorimetry

HP processes and the associated thermal history may induce structural changes of the polymer, such as a variation in the amount of crystalline phase. In order to investigate possible changes of crystallinity degree due to the HP treatment (i.e. combined effect of pressure and thermal history), a preliminary characterization was carried out by using Temperature-Modulated Differential Scanning Calorimetry (TMDSC) using a Q1000 apparatus (by TA Instruments, New Castle, DE, USA).

TMDSC technique offers improvements as compared to standard DSC in terms of sensitivity and resolution and allows the separation of overlapping events that are difficult or even impossible to distinguish by using a standard DSC (e.g. concurrent melting and re-crystallization phenomena). In fact, the modulation allows the separation of the total heat flow signal into its thermodynamic (heat capacity), reversible, component and its kinetic, irreversible, component.

To investigate the effect of pressure and temperature on PLA crystallinity, TMDSC was used to analyze untreated and HP treated PLA films. HP treated samples to be analyzed were cut from pouches containing the different foods. Moreover, to isolate the effect of temperature, TMDSC tests were also performed on two kinds of samples heated, respectively, at 90 °C for 5 min and at 120 °C for 20 min into an oven at atmospheric pressure, followed by natural cooling.

To perform the test, an amount of approximately 10–15 mg taken from untreated and treated samples, was sealed in nonhermetic aluminum pan and then tested under nitrogen atmosphere. Tests were performed in triplicate, for each treatment condition and type of food. Before conducting the experiments, a preliminary calibration with sapphire was performed. The heating scans were carried out from 30° to 180 °C at a heating rate of 2.5 °C/min, under a sinusoidal temperature modulation with a period of 40 s and a modulation of ± 0.265 °C.

In order to calculate the actual degree of crystallinity (χ_c) of the analyzed samples, the extra heat absorbed by the crystallites formed during heating in a TMDSC scan has to be subtracted from the total endothermic heat flow which encompasses both the heat associated to the melting of the crystallites originally present in the sample and that associated to the melting of the crystallites formed during the scan (Nam et al., 2001). In the TMDSC experiments, the endothermic heat flow, ΔH_{diff} , of the crystallites initially present in the sample can be easily calculated as $\Delta H_{\text{diff}} = \Delta H_{\text{rev}} - \Delta H_{\text{nonrev}}$, where ΔH_{rev} is the endothermic melting reversing enthalpy from the reversing heat flow trace and ΔH_{nonrev} is the exothermic ordering/crystallization (nonreversing) enthalpy from the nonreversing heat flow trace appearing, in the case of PLA films, in the temperature range 110–170 °C. In fact ΔH_{rev} is associated to the melting of all the crystals, while $-\Delta H_{\text{nonrev}}$ is associated to the melting only of crystals formed during the calorimetric scan.

The value of χ_c was then calculated as the ratio $\Delta H_{\text{diff}}/\Delta H_{100\%}$, where $\Delta H_{100\%}$ is the melting enthalpy of an ideal 100% crystalline phase of PLA that has been reported in the literature as being equal to 93.1 J/g (Fischer et al., 1973).

2.3.5. Dynamic mechanical analysis

Dynamic mechanical analysis (DMA) was also performed to gather information on the material structure as affected by HP treatment. The analyses were conducted using a DMA Q800 apparatus (by TA Instruments, New Castle, DE, USA). The tests were carried out in film tension mode (sample size 13.82 mm length, 5.5 mm wide, 0.045 mm thick) at a frequency of 1 Hz, in a temperature range from 0 to 120 °C, and the heating rate was 3 °C/min.

2.3.6. Density measurement

Density of glassy PLA can change as a consequence of HP treatments thus possibly promoting effects on mechanical and functional properties of packaging material. Density was determined by using a helium picnometer (AccuPyc 1340, Micromeritics, Norcross, GA, USA). The working principle of helium picnometry is based on the accurate determination of the volume of a sample of known weight, using a pressure decay technique. A sample of known mass is introduced into a cell of known volume then pressurized helium is expanded into the measuring cell from a reservoir chamber, so that the expanding gas occupies the entire volume of the cell that is not occupied by the sample. Based on a mass balance, on decrease of pressure and on the knowledge of reservoir and measuring cell volumes, the actual volume occupied by the sample can be determined. The density is hence calculated as the ratio of sample mass, evaluated using a Pioneer PA214C microbalance with a sensitivity of 10^{-5} g (by OHAUS, Parsippany, NJ, USA) and measured volume. The total accuracy of the density measurement is about 0.03% of the calculated value.

2.3.7. Gas permeability

Barrier properties on untreated material and changes brought about by HP treatment were determined for gases of interest in food packaging applications. In particular, evaluation of O₂ and CO₂ permeability was performed in a gas-film-gas configuration in dry conditions, using a custom made manometric permeabilimeter capable of working at several pressures and temperatures. The technique is based on the detection of the pressure increase at the downstream side of the polymer film pressurized at the upstream side. A detailed description of the equipment can be found in a previous paper by Nicodemo et al. (1992).

The polymer film to be tested (cut from pouches in the case of treated samples) was located in a gas-tight permeation cell separating the upstream and downstream chambers. Before each permeation test, the sample was allowed to desorb moisture, nitrogen, oxygen and other low molecular weight substances

possibly absorbed into the material, by degassing both chambers for a proper amount of time. At the start of the test, the upstream side was pressurized with the gas under investigation and the pressure in the downstream side, initially under high vacuum, started to increase, being monitored by a high sensitivity pressure transducer Baratron 121 (by MKS, Andover, MA, USA) with a 0.001 Torr sensitivity and an accuracy of 0.1% of the reading. From the measurement of this pressure increase and from the knowledge of volume of downstream compartment, the amount of gas permeated was evaluated as a function of time, thus allowing, when the permeation steady state was attained, the calculation of gas flux from the slope of the pressure vs. time curve. The permeability could then be calculated as the ratio of the mass flux divided by the driving force, represented by the ratio of pressure difference across the film and the film thickness.

2.3.8. Water vapor permeability

Barrier to water vapor is also of interest in food packaging applications. Water vapor transmission rate was measured using a Permatran W3/31 water vapor permeabilimeter (by Mocon Inc., Minneapolis, MN, USA). Measurements were carried out at 25 and 30 °C under different gradients of relative humidities: 0–30%, 0–50% and 0–90%.

2.3.9. Gas sorption isotherms

To complete the assessment of the effects of HP treatment on mass transport properties, gas sorption isotherms were also experimentally evaluated on treated and untreated samples. In particular, oxygen and carbon dioxide sorption isotherms were evaluated at several temperatures and sub-atmospheric pressures. This test consists in measuring the amount of gas absorbed at equilibrium into the polymer when in contact with a pure gas phase as a function of gas pressure. To this aim, it was used a custom made gas sorption system based on an electronic ultra-microbalance D200 (by CAHN Instruments, Madison, WI, USA) with a sensitivity of 0.0001 mg and an accuracy of ± 0.0002 mg. The information on gas solubility, coupled with gas permeability data, allowed for the calculation of average gas diffusivities as a function of concentration by using the following expression:

$$\bar{D}|_{c/2} = \frac{P}{S}$$

where $\bar{D}|_{c/2}$ is the average diffusivity in the range of gas concentration comprised between 0 and C, C being the equilibrium gas concentration measured by sorption isotherm into the sample at each pressure value (p). P is the measured permeability as evaluated when imposing the same value of pressure, as for sorption experiment, at the upstream side of the film in the permeation experiment. S stands for the 'secant' solubility calculated, at each experimental point of the isotherm, as the ratio C/p .

3. Results and discussion

3.1. Structural and morphological characterization

The multilayer structure of the PLA film can be inferred from DIC optical microscopy. Three layers are evident from collected images along the thickness of the sample (see Fig. 1a). The image was elaborated by an image processing software to identify the edges of layers and to measure their thicknesses (see Fig. 1b). The measured mean thickness of the whole film was about 45 μm while the mean thickness of the external layers was around to 6 μm each.

The structure of PLA was also investigated by using micro-Raman spectroscopy using as a reference Raman spectra reported in the literature (Furukawa et al., 2006; Kister et al., 2000). For

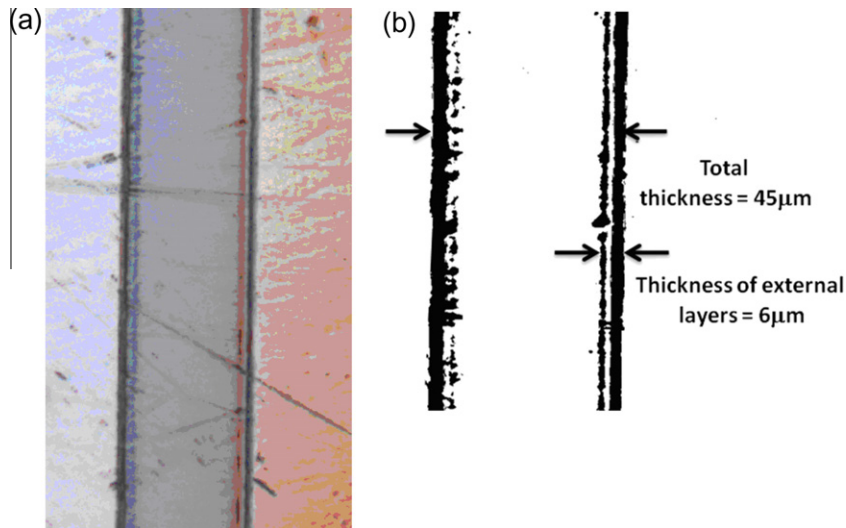


Fig. 1. Evidence of three layer structure of investigated PLA *Biophan 121* films (a) differential interference contrast image of a section of a film. (b) Picture of Fig. 1a after re-elaboration by using an image processing software.

comparison purposes and with the aim of quantifying the degree of crystallinity, measurements were first performed on an amorphous PLA (racemic PLA) film. The Raman spectra were collected in the region from 525 to 235 cm^{-1} ; this spectrum has been compared to the spectra of the semi-crystalline untreated, HP pasteurized and HP sterilized commercial PLA samples used in this study. In detail, difference of the relative height and intensity of the peaks at 418 and 400 cm^{-1} can be noticed among the samples.

Unfortunately, since reference samples of the investigated PLA at different known levels of crystallinity were not available, it was not possible to perform any calibration procedure that would allow to translate the spectroscopic parameter I_{418}/I_{400} in the degree of crystallinity.

Crystallinity profiles were collected, with a 1 μm resolution, starting from the edge to the center of the film on one side and from the center to the edge of sample on the other side to check for sample symmetry. All of the samples were found to be symmetrical (even the treated ones), hence, in the following, we will discuss only profiles performed from the external surface (i.e. the one exposed to pressurizing fluid, in treated samples) toward the center of the film thickness.

Results obtained by line scan from the edge to the center of the untreated PLA film in the 418–400 cm^{-1} region are reported in Fig. 2 (top plot): there is an initial region of about 2 μm where the sample appears to be completely amorphous. Afterwards, a steep increase of crystallinity is observed, encompassing a region of about 4 μm . Starting from $z = 6 \mu\text{m}$ the crystallinity profile becomes constant. This experimental finding confirms the results of DIC analysis which detected a three layer structure and clarifies the nature of the three layers. The external layer is likely due to the presence of a PLA with a different backbone structure as compared to the bulk material, as anticipated in Section 2.1.

The profile analysis performed on HP pasteurized and sterilized PLA containing tap water (see Fig. 2, center and bottom plots) showed a uniform crystallinity along the thickness. These spectroscopic findings, are also accompanied by the evidence of a marked opacification as well as of a considerable embrittlement that occur in the case of HP sterilized samples, independently of the type of packaged food, as witnessed by the picture reported in Fig. 3. The observed micro-Raman results, which are independent of the type of packaged food, could be tentatively ascribed to a diffusion of the external amorphous layer toward the bulk of the material favoured

by the processing conditions or to a further crystallization of amorphous regions under the action of pressure and heat (the latter only in the case of sterilization).

In fact, heat and pressure treatments are known to determine qualitative and quantitative changes of the crystalline phase. It is well known that thermal annealing of a semi-crystalline polymer at temperatures below the melting point can result in secondary crystallization, by acting on chains' mobility and alignment, narrowing the endothermic peak, increasing the crystallinity degree and promoting displacements of the melting peak towards higher temperatures. Also pressure is an important factor that can induce and accelerate crystallization of polymers. Morphology of crystalline phases, internal structure, phase diagrams as well as crystallization kinetics are all affected by pressure which has been found to promote a thickening process on crystal structure, producing extended chain lamellae. (Watanabe et al., 2003; He and Zoller, 1994; Liangbin et al., 2002; Law et al., 2008; Yu et al., 2008; Nakamura et al., 1972; Ito et al., 1995; Tsuji and Ikada, 1995; Weir et al., 2004).

Another important phenomenon which likely occurs when PLA is brought in contact with an aqueous environment is the possible occurrence of hydrolysis of macromolecules. Its occurrence supplies an alternative explanation for the evolution of crystallinity profiles observed by micro-Raman spectroscopy as a consequence of HP treatments. In fact the hydrolysis, beside promoting the breakage of ester bonds present on PLA backbone, could induce, under the synergic action of high pressure, a further crystallization of lower molecular weight macromolecules produced by the hydrolysis itself, thus justifying the change in crystallinity observed for the outer layers.

Numerous literature works (Ikada and Tsuji, 2000a,b; Tsuji and Ikada, 1995, 2003; Gupta and Deshmukh, 1982). Li et al. (1990a,b,c) report on degradation of PLA in an aqueous environment showing that it occurs through simple hydrolysis of ester bonds catalyzed by carboxylic groups, the hydrolysis rate increasing exponentially with degradation time. As a consequence of hydrolysis of ester linkage a carboxylic acid is formed on one end and an hydroxyl group are on the other. The degradation process proceeds first by water diffusion within the amorphous regions, which are less organized and allows water to penetrate more easily than the highly ordered densely packed crystalline regions, then hydrolytic degradation of the amorphous regions follows, the degradation rate

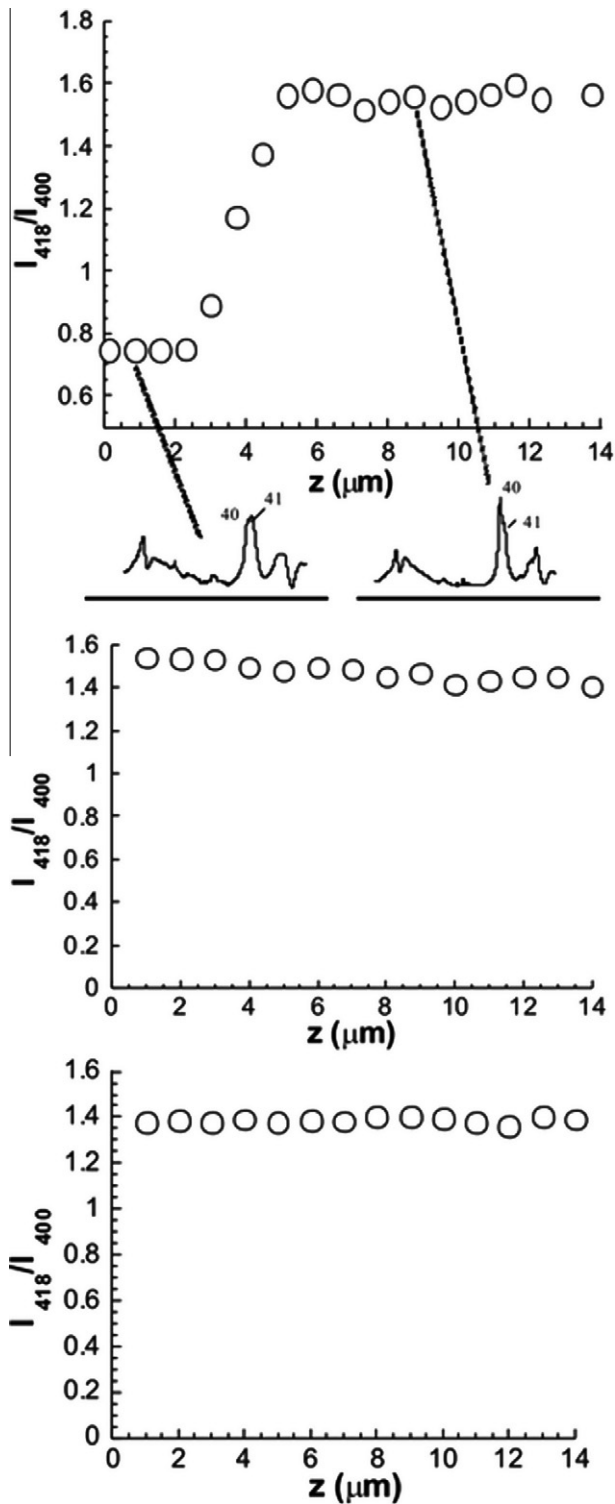


Fig. 2. Profile analysis of untreated and HP treated PLA *Biophan 121* film as determined from the edge to center of the film thickness using confocal Raman microscopy. The plots highlight the differences in crystallinity profiles within the samples. Top: untreated film; center: film after pasteurization at 700 MPa (tap water as packaged foodstuff); bottom: film after sterilization at 700 MPa (tap water as packaged foodstuff).

increasing with temperature. This rate increase becomes significant above the glass transition temperature of the polymer. Tsuji et al. (2001) tested PLLA films in buffers at 97 °C and showed that amorphous regions crystallized while degrading thus slightly slowing down the rate of hydrolysis. Crystallization is reported to be



Fig. 3. Evidence of opacification and embrittlement of HP sterilized PLA film. Photograph of a PLA *Biophan 121* film after HP sterilization of a pouches containing solid carrots as packaged foodstuff.

primarily due to the degradation of the amorphous regions, being also related to a reorganization of loose chain ends (Tsuji et al., 2001; Mathisen et al., 1991; Hakkarainen et al., 1996). In fact, due to the bonds breaking, the lower molecular weight chains gain chain mobility, reorganizing to increase the overall degree of crystallinity in the polymer.

On the basis of the mentioned literature results, the micro-Raman experimental outcomes reported for HP treated PLA samples could be also interpreted as reflecting the crystallization phenomena of smaller molecular weight species formed by the hydrolysis of PLA determined by the action of water, which was used as pressure transmitting medium or is largely present in the packaged foodstuff. This phenomenon is expected to be more relevant in the two external, initially amorphous, layers of PLA films, exposed, respectively to the external pressurizing fluid and to the packaged foodstuff. High pressure conditions imposed during treatment are likely to further enhance this crystallization phenomenon as compared to the simple exposition to liquid water at atmospheric pressure.

To confirm the occurrence of hydrolysis during the relatively short time of HP treatment, ATR-FT-IR spectra were obtained for untreated, pasteurized and sterilized PLA and are shown in Fig. 4. Collected spectra refer to the surface of treated films exposed to the foodstuff. In the HP treated samples a new absorption occurs at about 1610 cm^{-1} , this band is characteristic of the carbonyl stretching of a carboxylate group and indicates the occurrence of hydrolysis, and the shape and the intensity of the band change as a function of the HP treatment. This intensity is more pronounced

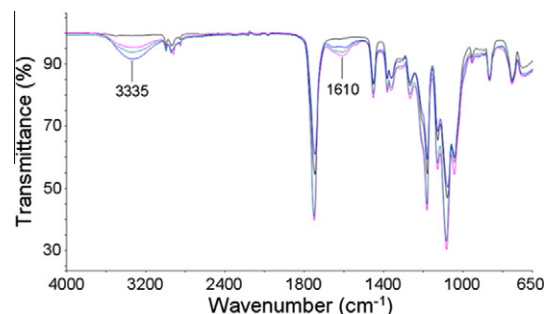


Fig. 4. Assessment by ATR-FT-IR analysis of the occurrence of hydrolysis in the proximity of the surface of HP treated PLA *Biophan 121* films exposed to foodstuff. ATR-FT-IR spectra of untreated PLA *Biophan 121* film (black) compared to pasteurized PLA at 700 MPa used to package solid carrots (blue), sterilized PLA at 700 MPa used to package tap water (pink) and solid carrots (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

for sterilized sample as compared to pasteurized and untreated film. New features are also observed in OH stretching region. Here a broad absorption centered at about 3335 cm^{-1} is observed, which can be related to the formation of hydroxyl groups and/or to the surface sorption of atmospheric moisture as a consequence of the improved hydrophilicity of the sample surface after hydrolysis. This possible interference of absorbed water could be avoided by performing ATR experiments under vacuum or dry atmosphere. However we did not performed this analysis, since the purpose of the ATR test was just to supply a qualitative proof of occurrence of hydrolysis under the adopted HP treatment conditions. In fact, a deeper investigation aimed at a quantitative assessment of this phenomenon would imply also the application of careful calibration and normalization procedures.

The spectra collected for the film surface exposed to the pressure transmitting fluid, also displayed a similar behavior, with evidence of hydrolysis occurring in HP pasteurized and, to a greater extent, in HP sterilized sample. Finally, within the limits of the qualitative analysis performed, no systematic effect of the type of food on the extent of hydrolysis has been observed.

Further information on the structure of the starting material, and on its possible modification induced by HP treatments, has been obtained by polarized FT-IR spectroscopy. In particular, this spectroscopic technique has been used to study the effects of treatment on the in plane orientation of the film. Peaks characteristic of the amorphous and crystalline phases were identified at 955 and 920 cm^{-1} , respectively (Meaurio et al., 2006).

As demonstrated in Fig. 5, the above peaks are suitable to evaluate the orientation degree of the amorphous and crystalline phases. In particular, the orientation parameter R (dichroic ratio) has been calculated as

$$R = A_{\parallel}/A_{\perp}$$

where A_{\parallel} is the absorbance area measured with radiation polarized parallel to the machine direction (MD) of the film and A_{\perp} is the corresponding measurement with polarization perpendicular to MD.

The peaks at 955 and 920 cm^{-1} were respectively used to evaluate the orientation of the amorphous and crystalline phases. In all cases the orientation is confined to the crystalline phase only. The untreated sample has been found to be highly oriented in the MD ($R = 12$, see Table 2). The HP pasteurized PLA samples display significantly lower orientation ($R = 7$, see Table 2), while for the HP sterilized samples higher R values were detected ($R = 21$, see Table 2). The lowering of orientation observed for HP pasteurized is likely related to a partial isotropization of the crystalline domains as a consequence of the increased mobility of the interconnecting

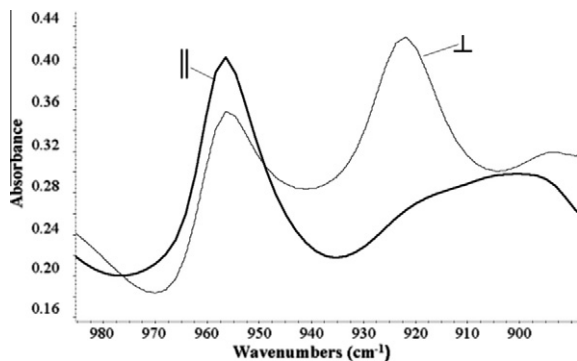


Fig. 5. Assessment of the effect of HP treatment on the orientation of the amorphous and crystalline regions of PLA Biophan 121 film. The case of FT-IR spectra collected for a film used to package solid carrots, sterilized at 700 MPa is reported. Spectra collected with parallel polarized radiation (\parallel) and with perpendicularly polarized radiation (\perp).

Table 2
Values of the orientation parameter.

Sample	R (from peak areas)
Untreated PLA	12
Pasteurized PLA at 700 MPa used to package tap water	7
Sterilized PLA at 700 MPa used to package solid carrots	21

amorphous phase brought about by the temperature increase (up to $40\text{ }^{\circ}\text{C}$) and by the plasticizing action of sorbed water. Conversely, the increase of orientation detected in the case of HP sterilized samples, could be tentatively related to the orientation of the crystalline domains formed in the external layers as a consequence of the pressure-induced re-crystallization process.

3.2. Calorimetric and dynamical–mechanical analysis: glass transitions, crystallinity and tensile moduli

Further insight into the sample structure has been gained by performing TMDSC. In the case of untreated PLA (see Fig. 6a and b), the calorimetric analysis (non-reversing heat flow trace) indicates the presence of two glass transition temperatures (T_g), respectively at 55.8 and $62.4\text{ }^{\circ}\text{C}$. Both glass transitions are immediately preceded by an endothermic peak, placed at the onset of the transition, which is due to enthalpy relaxation associated to sub- T_g aging of the polymer. The first transition, at $55.8\text{ }^{\circ}\text{C}$, is likely associated to the amorphous external layers and the second, at $62.4\text{ }^{\circ}\text{C}$, to the PLA forming the inner core of the film. The T_g of the inner core is higher due to the fact that the presence of crystals

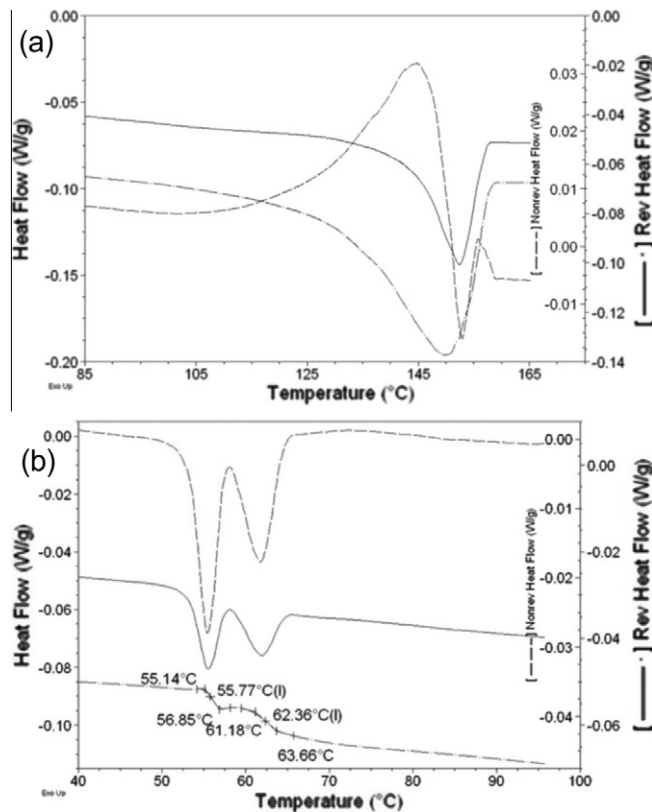


Fig. 6. TMDSC scans of untreated PLA Biophan 121 film. The heating scans were performed under sinusoidal temperature modulation with a heating rate of $2\text{ }^{\circ}\text{C}/\text{min}$. (a) Modulation parameters optimized to highlight melting/recrystallization phenomena, a period of 80 s and a modulation of $\pm 0.424\text{ }^{\circ}\text{C}$ between 70 and $170\text{ }^{\circ}\text{C}$. (b) Modulation parameters optimized for highlighting glass transition region, with a heating rate of $2.5\text{ }^{\circ}\text{C}$, a period of 40 s and a modulation of $\pm 1.06\text{ }^{\circ}\text{C}$.

determine a constraint that reduces the mobility of the amorphous phase (Jamshidi et al., 1988; Fukushima and Kimura, 2006; Tsuji and Ikada, 1999; Urayama et al., 2003; Narladkar et al., 2008). Data for T_g 's are reported in Table 3. Tests were performed in triplicate for each type of sample and in Table 3 are reported the minimum and maximum values detected. These evidences are confirmed by dynamical–mechanical tests performed on the film (see Fig. 7) as a function of temperature. The presence of the two glass transitions is indicated by two peaks in the loss modulus trace and two regions of decrease of storage modulus, roughly located at the same temperature values as detected by TMDSC.

The degree of crystallinity was estimated according to the procedure described in Section 2. The estimated χ_c for untreated PLA is reported in Table 3: this value is obviously an average of the crystallinity of the three layers composing the film. Since the tests have been performed in triplicate for each type of sample, in Table 3 are reported the minimum and maximum value which have been measured.

Before analysing the consequences of HP treatments, the separate effects of solely exposing samples to the treatment temperatures in air dry and in liquid water at atmospheric pressure have been investigated to gain a better insight into the phenomena occurring during treatments, thus isolating the contribution of each variable (i.e. temperature, pressure, nature of pressure transmitting fluid).

Liquid water is present in the external phase, being the pressure transmitting fluid, and is present, in different concentrations, inside the package since packaged foodstuff contain a considerable percentage of water. PLA is a hydrophobic polymer, which absorb small amounts of water, without dissolving or swelling in an aqueous environment. Besides the mentioned involvement in hydrolytic reaction, another relevant effect of absorbed water is the polymer plasticization. In fact, the presence of water results in a significant reduction of the glass transition temperatures of the polymer. This effect is evident both in TMDSC and DMA traces. As an example, in Fig. 8, are reported DMA scans for 'dry' and 'wet' (i.e. equilibrated with liquid water at room temperature) PLA films. It is evident how the main T_g (i.e. that of the inner core layer) is located at 69.3 °C for dry PLA and at 60.0 °C for 'wet' PLA. A corresponding reduction, from 60.7 to 51.8 °C, is also evident for the lower T_g attributed to external amorphous layers. This 10 °C depression of T_g is actually even underestimated: in fact, during the temperature scan of the DMA test, part of the water originally absorbed into the 'wet' sample has been desorbed. Consequently, the plasticizing effect is expected to be even higher. In fact the values for the glass transition temperatures presented in Figs. 6b and 7 for untreated samples are intermediate between the dry and wet samples since the standard test procedure performed usually for TMDSC and DMA analyses does not allow a complete removal of sorbed water.

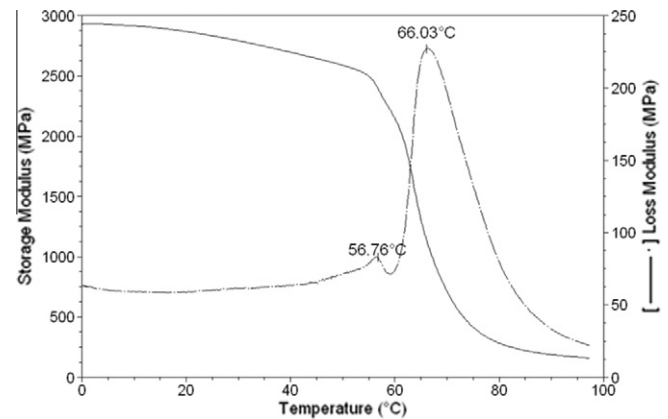


Fig. 7. Dynamical–mechanical tests performed on untreated PLA *Biophan 121* film to highlight glass transitions. Loss modulus and storage moduli are reported vs. temperature, at a scan rate of 3 °C/min.

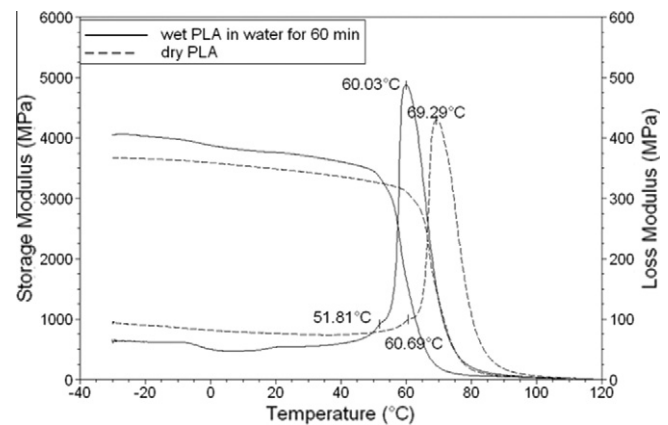


Fig. 8. Evidence of plasticization of untreated PLA film by sorbed water after equilibration in liquid water at room temperature. In the plot are reported the loss modulus traces and storage modulus curves from DMA scans of dry and 'wet' untreated PLA, performed at a scan rate of 3 °C/min.

In order to assess if a simple treatment at atmospheric pressure in liquid water determines any irreversible effect on PLA crystallinity and T_g 's, DMA and TMDSC analysis have been performed on samples treated in water at 40 °C for 5 min and at 90 °C for 15 min. It is important to notice that, prior to performing the calorimetric and dynamical–mechanical tests, samples have been re-equilibrated with ambient humidity. In Table 3 are reported, in particular, the results of TMDSC analysis which indicates that there is no significant change in the value of T_g 's, while a small increase

Table 3

Degree of crystallinity, χ_c , of untreated and HP treated PLA *Biophan 121* film (results reported for treated samples refer to packages containing tap water).

PLA sample	χ_c	Low and high T_g (°C) ^a	
Untreated	25.3–25.8	55.6–56	62.1–62.8
Aged at 40 °C in air, 5 min	25.1–25.6	55.2–55.7	62.5–66.9
Aged at 40 °C in water, 5 min	26.6–27.1	55.2–55.6	62.8–63.1
Aged at 90 °C in air, 15 min	26.2–26.5	54.8–55.4	61.7–62.2
Aged at 90 °C in water, 15 min	27.5–28.0	55.0–55.5	61.8–62.3
Pasteurized at 200 MPa (tap water)	24.9–25.4	54.2–54.5	62.0–62.5
Pasteurized at 700 MPa (tap water)	24.2–24.7	55.4–55.8	62.0–62.5
Pasteurized 700 MPa (solid carrots)	26.4–26.9	55.0–55.3	61.7–61.9
Pasteurized 700 MPa (carrot juice)	25.2–25.7	55.1–55.6	62.6–63.0
Sterilized 700 MPa (tap water)	29.2–29.6	58.6–59.1	63.4–64.0
Sterilized 700 MPa (solid carrots)	29.3–29.7	The two transitions are overlapped	

^a The ranges reported indicate the lowest–highest value detected for each type of sample in triplicate tests.

in crystallinity level is detectable, likely associated to a partial crystallization of the outer layers of PLA films. Moreover, the sample treated at 90 °C displays also a development of opacity, which has been attributed to hydrolysis phenomena involving the surface region of the samples.

The effect of a simple treatment in dry air at atmospheric pressure and temperatures equal to 40 and 90 °C has also been assessed on PLA films and quantitative results of TMDSC analyses are again reported in Table 3. No effect was observed in the first case, while in the case of sample treated at 90 °C it was detected a slight increase of crystallinity (in the range of 1%). This increase, however, is lower than in the case of treatment in liquid water at both 40 and 90 °C where an increase of crystallinity ranging from 1.4% to 2.2% was detected (see Table 3), since water is able to plasticize the PLA matrix thus kinetically favouring the crystallization process and, as already evidenced, is capable of hydrolytic attack which, in turn, reduces molecular weight of polymer backbones and further promotes crystallization. No opacification was noticed for samples treated in dry air, both at 40 and 90 °C.

In the case of HP treated films, TMDSC and DMA measurements performed on pasteurized samples revealed that HP pasteurization did not promote any significant effect on the glass transition temperatures and on weight fraction of crystallinity (see Table 3 for the results of TMDSC analysis). No trends can be evidenced from the analysis of the experimental results of the DMA analyses, but it can be concluded that the treatment did not compromise the mechanical properties, at least in terms of tensile modulus.

In the case of HP sterilized PLA films, only TMDSC measurements were performed since it was impossible to run reliable DMA analyses in view of the brittleness of the treated films. Inspection of the TMDSC traces indicated that the lower T_g , attributed to external layers, increased after treatment getting closer to the value of the higher T_g (see, for example, TMDSC trace reported in Fig. 9 for the case of sample sterilized at 700 MPa, with tap water as packaged food). This increase is roughly proportional to the pressure applied during treatments and occurred with all of the packaged foods (see Table 3). Concerning crystallinity, TMDSC analysis indicates that sterilization treatment produced, at all investigated treatment pressures, an overall increase of crystallinity in the PLA films (see Table 3), which could be ascribed to partial re-crystallization of the external layers induced by hydrolytic action of water in contact with the film and the high pressure conditions. The increase of crystallinity and the hydrolysis of macromolecular backbones promoted by HP sterilization made the material more brittle and was accompanied by a significant opacification. These effects on PLA film prevent its use for HP sterilization. A similar behavior has been detected for samples

simply immersed in tap water at 90 °C at atmospheric pressure, although, in this case, the opacification effect was less pronounced.

The increase of T_g of external layers brought about by HP sterilization is consistent with the hypothesized crystallization of the originally amorphous external layers. In fact, crystalline regions can act as mobility constraint for the macromolecules in the amorphous phase, thus determining the observed increase in T_g . Smaller, if not negligible, changes were instead observed for the higher T_g , thus pointing to the conclusion that there were not significant changes in the structure of the inner layer of PLA film.

Taken together, the results of spectroscopic, calorimetric and dynamical mechanical analysis, indicate that: (a) in the case of HP pasteurized sample, the treatment has promoted some morphological rearrangement, mainly consisting in a diffusion of the external amorphous layer toward the bulk of the material favoured by the processing conditions, and some limited hydrolysis of the surface layers of PLA films; (b) in the case of HP sterilized films, in addition to the mentioned morphological rearrangement, the sorption of water at higher temperatures is accompanied by a significant hydrolysis, which reduces the average molecular weight of macromolecules thus increasing mobility and favouring further crystallization of the system, this crystallization process being also thermodynamically favoured by the high pressure conditions.

3.3. Density evolution

Another important possible effect of HP treatments is the change of density of the amorphous regions of PLA samples. In fact, PLA is a glassy polymer at room temperature and, as it is well known (Koros and Hellums, 1990), the density of a glassy polymer strongly depends upon its previous temperature and pressure history below the glass transition temperature, which has itself a pressure-dependent value. In fact, samples submitted to HP pasteurization and sterilization experience complex sub- T_g annealing as well as heating up to a rubbery state above the current T_g . As a consequence, depending on actual pressure/temperature history, density of amorphous regions evolve in a rather predictable way. This issue has been specifically addressed for PLA *Biophan 121* film in a recent contribution (Grassia et al., 2011) on the basis of a theoretical model developed for predicting free volume evolution in glassy polymers.

The change of density of the amorphous phase is of interest since it reflects changes in the amount of free volume which, in turn, do affect several properties, including mechanical properties and gas/vapor permeability and solubility. It should be emphasized that, in view of the measuring technique, only the overall change in density of semicrystalline polymers is accessible experimentally. The measured changes in density could then result both from changes in free volume of amorphous regions and from change of the amount of crystallinity or from changes of the structure of crystalline lattice induced by pressure.

Density has been determined at 28.4 °C for untreated PLA film and for several treated PLA samples and results are reported in Table 4.

As can be deduced from the previous discussion, it is a complex matter to unequivocally attribute density changes to a specific process involving the crystalline and/or the amorphous regions. Since the total density measured for the samples is actually a weight average of the density of the crystalline phase and of the amorphous phase, both depending on sample history, the density of pasteurized sample, which always remain during treatment below the actual T_g , can be either higher or lower than the one of the untreated ones, depending on the prevailing effect. Conversely, in the case of sterilized samples, the increase of crystallinity seems to be the dominating effect, resulting in a net increase of density. The multilayer structure of the films further complicate the analysis.

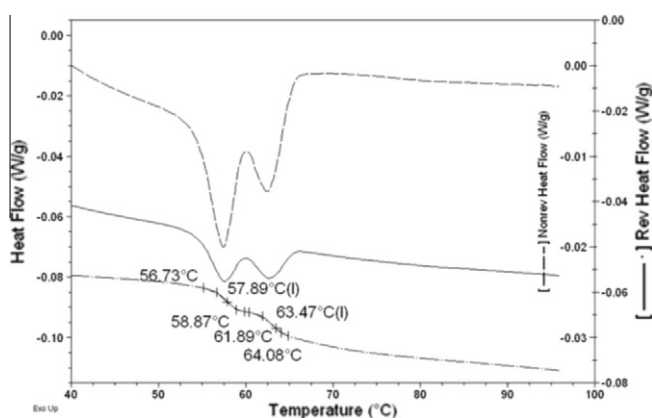


Fig. 9. Calorimetric analysis performed on an HP sterilized PLA *Biophan 121* film highlighting the glass transition regions. TMDSC scans performed at a heating rate of 2.5 °C, a period of 40 s and a modulation of ± 1.06 °C at 700 MPa. Tap water as packaged foodstuff.

Table 4

Determination of density at 28.4 °C of untreated and HP treated PLA *Biophan 121* film for different packaged foodstuff.

PLA samples	ρ (g/cm ³)
Untreated	1.2640 ± 0.0002
Pasteurized 200 MPa (tap water)	1.2647 ± 0.002
Pasteurized 500 MPa (tap water)	1.2646 ± 0.0002
Pasteurized 700 MPa (tap water)	1.2642 ± 0.0002
Pasteurized 200 MPa solid carrots	1.2675 ± 0.0003
Pasteurized 500 MPa solid carrots	1.2655 ± 0.0002
Pasteurized 700 MPa solid carrots	1.2608 ± 0.0003
Sterilized 200 MPa (tap water)	1.2680 ± 0.0002
Sterilized 700 MPa (tap water)	1.2740 ± 0.0004

3.4. Sorption thermodynamics and mass transport properties

The investigation of the effect of HP treatments on sorption thermodynamics and mass transport properties of gases (O₂ and CO₂) and water vapor, besides being of interest per se in food packaging applications, is also useful to gather further information on structural and chemical characteristics of the samples. In fact crystallinity amount and density of the amorphous phase of the polymer both affect gas transport and sorption properties. In the following we first discuss the results obtained for HP pasteurized samples. Subsequently few results are presented for HP sterilized samples: in this case, in view of the strong alterations induced by treatment, it was not possible to perform permeability tests, and only sorption tests were performed.

Gases and water vapor permeability and solubility have been measured before and after HP treatments. A crystallinity increase is expected to induce a reduction of solubility and permeability. In fact, an increase of crystallinity determines an increase in tortuosity of the diffusive paths thus promoting a decrease of diffusivity and, at the same time, a decrease of solubility, being the crystalline domains generally impervious to small molecular weight penetrants. On the other hand, a decrease of density of the amorphous phase induces an opposite effect, promoting an increase of solubility and permeability related to the increase of the available free volume.

The experimental carbon dioxide and oxygen permeability values for some of the pasteurized PLA samples are reported in Table 5. These results indicate that the effects of HP pasteurization on carbon dioxide and oxygen permeabilities are negligible. The morphological changes discussed before, consisting in a slight decrease of density of the amorphous regions of the samples, are not significant enough to bring about detectable changes of the mass transport properties. This conclusion is also confirmed by the very limited effect of HP pasteurization on carbon dioxide solubility (see, as an example, the CO₂ sorption isotherm at 20 °C for PLA after pasteurization at 700 MPa, tap water as packaged food, reported in Fig. 10) and diffusivity (see, as an example, Fig. 11 reporting dependence of carbon dioxide diffusivity on its concentration, at 20 °C for PLA after pasteurization at 700 MPa, tap water as packaged food).

Water vapor permeability of pasteurized PLA (see Table 6) has been found to be moderately smaller if compared to the case of

untreated PLA. In this case, structural changes seems to be responsible for a decrease in water solubility which determines, in turn, an decrease in permeability.

All the results discussed above for HP pasteurized samples were found to be independent on the type of packaged food.

Although the main focus of the present contribution is on the effects of HP treatments on PLA properties and no analyses were actually performed to assess the specific performances in terms of shelf-life of the packaged foods, we can however briefly address the problem of preservation of the investigated foodstuff in terms of packaging requirements. In fact, packaging material to be used for carrots in the different forms, should be suited to guarantee appropriate levels of humidity and of concentration of oxygen and carbon dioxide in the package headspace. Commonly used packaging materials for packaging of minimally processed carrots and carrot juice are multilayer structures obtained by combining bioriented polypropylene and low density polyethylene (BOPP/LDPE) and nylon ethylene vinyl acetate (nylon/EVA). These structures have been taken here as a reference for a comparison in terms of gas transmission rates, which indicates that PLA films display intermediate properties. In fact oxygen and carbon dioxide permeabilities are lower than in the case of BOPP/LDPE films but higher than in the case of the nylon based structures; moreover the ratio of carbon dioxide and oxygen permeabilities for the case of PLA have values which are close to those of the other two multilayer structures. Conversely, water vapor transmission rate in the case of PLA is higher than both the other types of films. However, this is to be considered as a typical drawback of biodegradable polymers even though, within this class of polymers, PLA is one of the materials showing lower water permeabilities. These barrier properties of PLA are, for all practical purposes, unaffected by HP pasteurization. On this basis, PLA can be considered as a possible candidate for packaging of HP pasteurized sliced carrots, carrot juices and puree.

Passing to the case of HP sterilized samples, as anticipated, it was not possible to perform reliable permeation experiments, in view of the irregular shape of the treated samples. Only sorption isotherms were evaluated the changes in solubility were not found to be very significant. However, analysis of sorption isotherms supplied some information on polymer structure after treatment. In fact, in the case of sterilized samples (as an example, see Fig. 10 for the carbon dioxide sorption isotherm at 20 °C, for the case of sample sterilized at 700 MPa, tap water as packaged food), a limited but detectable increase of solubility has been found. It can be hypothesized that, although the crystallinity of the outer regions of the film is increased as a consequence of HP treatment, the treatment promotes also a decrease of density of the amorphous phase, since, during treatment, the sample is brought above its T_g , thus inducing a 'rejuvenation' of the amorphous region of the sample with associated increase of specific volume of the noncrystalline phase. Carbon dioxide diffusivities, evaluated from sorption experiments (as an example, see Fig. 11 for the carbon dioxide diffusivity at 20 °C), increased with carbon dioxide concentration, as expected in glassy polymers, and were the lowest in the case of sterilized PLA. This is due to two concurrent causes: (a) a higher amount of crystalline phase is present in sterilized samples,

Table 5

Oxygen and carbon dioxide permeability expressed in [(cm³ (STP) cm)/(cm² s atm)], for untreated PLA and for PLA used to package tap water, after Pasteurization at 700 MPa calculated at 1 atm at several temperatures.

T (°C)	CO ₂ untreated	CO ₂ HP pasteurized	O ₂ untreated	O ₂ HP pasteurized
33	1.0824e-08	1.1749e-08	2.7132e-09	2.9009e-09
36	1.2270e-08		2.9938e-09	
40	1.3536e-08	1.3250e-08	3.4906e-09	3.4944e-09
44	1.6026e-08		4.0783e-09	
48	1.8161e-08	1.7265e-08	4.6438e-09	4.5665e-09

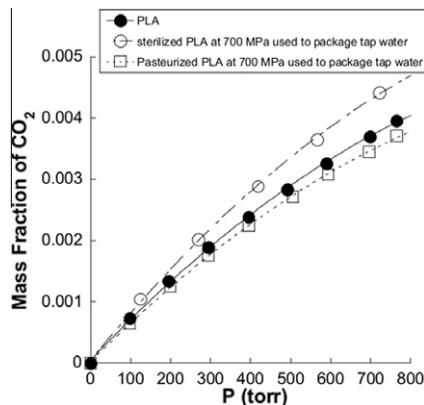


Fig. 10. Effect of HP treatment of carbon dioxide solubility in PLA Biophan 121 films. CO₂ solubility at 20 °C is reported vs. pressure for HP pasteurized and sterilized PLA at the pressure of 700 MPa (tap water as packaged foodstuff) as compared to untreated PLA.

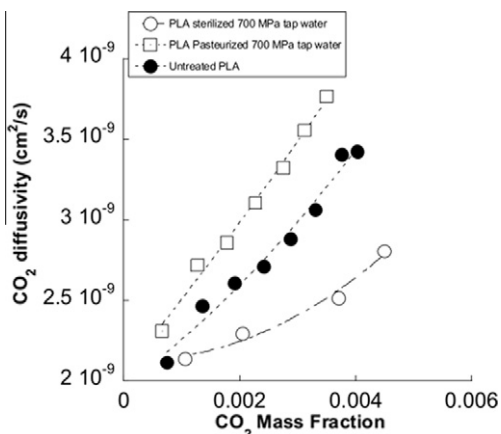


Fig. 11. Effect of HP treatment on CO₂ diffusivity in PLA Biophan 121 films. CO₂ diffusivity at 20 °C is reported vs. carbon dioxide mass fraction for HP pasteurized and sterilized PLA at the pressure of 700 MPa (tap water as packaged foodstuff) as compared to untreated PLA.

Table 6

Water vapor permeability of untreated PLA compared to PLA pasteurized at 700 MPa (foodstuff: carrot juice).

Sample	P (g cm/(cm ² atm s))			
	T (°C)	R.H. (%)		
		30	50	90
Untreated PLA	25	1.4846e-08	1.4722e-08	1.3802e-08
	30	1.3818e-08	1.4000e-08	1.3988e-08
Pasteurized PLA at 700 (MPa) carrot juice	25	1.2711e-08	1.2235e-08	1.2782e-08
	30	1.1937e-08	1.1439e-08	1.2186e-08

determining an increase of tortuosity of diffusive paths as compared to untreated and HP pasteurized samples; (b) 'excess' free volume of glassy PLA after HP sterilization was increased in the amorphous regions of the sample (see the increase in sorption capacity of carbon dioxide), thus promoting, as hypothesized in several mass transport theories (Koros and Hellums, 1990), a decrease of diffusivity as compared to the case of untreated and HP pasteurized samples, since excess free volume packets act as adsorbing sites that slow down the diffusion.

4. Conclusions

PLA has attracted the attention of industry as a packaging material because, in addition to its biodegradability characteristics, it exhibits good mechanical, physical and barrier properties. In this investigation we have analyzed the suitability of commercial flexible packaging films of PLA for use in HP pasteurization and sterilization.

This investigation indicates that this material is able to withstand HP pasteurization treatments without displaying any relevant decrease of structural and functional properties. In fact, HP pasteurization treatments, performed at pressures ranging from 200 to 700 MPa, did promote some degree of hydrolysis and limited effects on the morphology and structure of the material, without affecting mechanical and barrier properties of treated PLA films as compared to the untreated polymer.

On the other hand, PLA films were found to be unsuitable for high temperature (90 °C and higher) HP sterilization. In fact, sterilization conditions determine the hydrolysis of outer layers of the film inducing also an increase of crystallinity that is associated with unacceptable embrittlement and opacification of the material. Sterilization promoted an increase of density of the sample as a whole (associated to crystallization), and, at the same time, a decrease of density of the amorphous phase. Limited changes were observed for the mass transport properties. Overall, these results indicate that PLA films cannot be used in packaging application where HP sterilization processes are to be performed.

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