

Special Issue: Bio-based Packaging

Guest Editors: José M. Lagarón, Amparo López-Rubio, and María José Fabra
Institute of Agrochemistry and Food Technology of the Spanish Council for Scientific Research

EDITORIAL

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Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) films for food packaging: Physical-chemical and structural stability under food contact conditions

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ABSTRACT: The objective of the present article is to bring new insights into the relationships between the structural and physical-chemical stability and the inertness of PHBV films with respect to the targeted food packaging application. It is concluded from overall migration tests that PHBV films can be used as food contact materials for any type of food. Functional properties of PHBV films (mechanical properties and water vapor permeability) were also very stable after contact at 40 C during 10 days with all food simulating liquids tested (water, acetic acid 3% (w/v), ethanol 20% (v/v), and iso-octane), except with ethanol 95% (v/v). Ethanol 95% (v/v) was identified as the worst case for PHBV films, with a high sorption value and an increase in the water vapor permeability. This was mainly explained by a significant plasticizing effect, together with a decrease in both the molecular weight and the crystallinity degree of PHBV films. From an industrial point of view, it allows assessing that the packaging functions of PHBV are fulfilled all over the food supply chain.   2015 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2016**, *133*, 41850.

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INTRODUCTION

In addition to its role of storing food, the main function of food packaging is to ensure food quality and safety, i.e. to prevent physical, chemical, physiological, and microbial spoilage of foodstuff, from its production until its consumption, by controlling mass transfers of many types of low molecular weight molecules. Transport properties of materials must be evaluated not only for gases and vapors between the environment and the food product through the packaging materials, but includes also consideration about a mass transfer process of components from the packaging toward the food and vice versa. This latter process is called “food/packaging interaction” and includes the so-called “chemical migration” (migration of chemical contaminants packaging components toward the food).^{1,2} Among the materials available to pack food, plastics are versatile materials displaying the largest attractiveness, mainly due to their large panel of mechanical, optical, and barrier properties. However, plastics, which are for the greater part from fossil origin and represent almost 40% of the general plastic demand, generate many environmental concerns, including a contribution to the depletion in petroleum resources and waste management issues. Bioplastics, defined as bio-sourced and/or biodegradable materi-

als, were extensively studied this last decade to propose alternative materials designed to present a better environmental impact than conventional plastic materials.³ Among the promising bioplastics, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), which is a bacterial thermoplastic linear aliphatic polyester, appears as very attractive since it is fully biodegradable in composting, backyard or landfill conditions^{4,5} and can be produced from food industry by-products.⁶

One critical key point in food application of biodegradable packaging is the sensitivity balance in the respect that such material should be durable enough for protection, but still mid-term biodegradable.⁷ The structural and physical-chemical stability of packaging materials under their whole life cycle is consequently the main issue to ensure their ability to preserve food quality and safety within recommended usage conditions. Substances arising from physical-chemical material degradation as well as breakdown products and/or intentionally added additives such as antioxidants or plasticizers could migrate during the contact of the materials with the food. The inertness of PHBV-based materials has already been evaluated through overall migration tests using liquid food simulants.^{8–11} However, knowledge is still missing on the dependency of the material

inertness (evaluated through overall migration tests) with the structural and physical–chemical stability of biodegradable materials. If moisture, temperature, and mechanical stress are well-controlled parameters, other ones such as food acidity, water activity or fat content of the food can also impact in different ways the functional properties (including barrier and mechanical properties) of biodegradable materials set in contact.^{1,12–14} So far, although PHBV-based materials are authorized for food contact applications by the European Plastics Regulation (EU) No 10/2011, hardly no data is available on the relationships between their chemical safety and physical–chemical and structural stability under food contact conditions. Such information would be of prime importance not only for understanding purposes, but also at the industrial level, to ensure that the packaging functions are fulfilled all over the food supply chain.

In this context, the objective of the present article is to bring new and necessary insights into the relationships between the structural and physical–chemical stability and the inertness of PHBV-based materials with respect to the targeted food packaging application. For that purpose, the influence of severe, yet realistic conditions of storage and food contact on the evolution of their sorption behavior, mechanical, and barrier properties, as well as on the overall migration, was investigated. The measurement of the overall migration was performed under standardized testing conditions and the results were discussed in relation to the structural stability of materials, which was evaluated using complementary methods, including thermal transition temperatures (DSC and TGA), degree of crystallinity (DSC), distribution in molecular weight (GPC), and film morphology (SEM observations).

EXPERIMENTAL

Materials

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) PHBV (3% HV) in pellets form (ENMAT Injection Molding Grade Y1000P) was provided by Tianan Biologic Material (China). As reported by the manufacturer, PHBV contains less than 0.5% (w/w) of nucleating agents (boron nitride) and about 1% (w/w) of antioxidants (Irganox[®] 1010). Acetic acid (C₂H₄O₂, purity >99.9% (w/v), Carlo Erba), ethanol (CH₃CH₂OH, anhydride; Api), and iso-octane (C₈H₁₈, purity >99.0%, Sigma-Aldrich) and olive oil were used to prepare the food liquid simulants (FSLs) for overall migration tests.

Preparation of PHBV Films

PHBV films were prepared by extrusion to simulate the packaging process using a lab-scale twin-screw extruder with a L/D = 40 and a screw diameter of 16 mm (Eurolab from ThermoFisher Scientific). A single screw volumetric feeder, 16 mm (ThermoFisher Scientific) was used for feeding the polymer pellets, the temperature profile from the polymer feeding to the die varied from 180 to 160°C. After the extrusion, the material was cooled with cold water and dried with compressed air immediately. The PHBV was finally cut into pellets by a pellet-

izer with a 2–3 mm length and dried in an oven at 70°C for 24 h.

In a second step, films (average thickness = 0.25 mm) were prepared by hot-pressing the pellets between two Teflon coated plates at 170°C in a hydraulic press (PLM 10 T, Techmo, Nazelles, France). The samples were first allowed to melt for 3 min, and then, a pressure of 150 bars was applied for 2 min. Finally, samples were cooled at room temperature and dried in a vacuum oven at 70°C.

Characterization of Materials

Sorption of Liquid Food Simulants. The weight uptake of FSLs by films was gravimetrically determined using a precision balance (10⁻⁴ g). After the incubation at 40°C for 10 days, 60 cm² of film samples were weighted immediately after having wiped carefully 3 times the excess of FSLs with tissue paper. FSLs sorption was calculated as follows:

$$\text{FSL sorption (mg.g}^{-1} \text{ d.b.)} = \frac{m_t - m_f}{m_f} \quad (1)$$

where m_t is the weight of the film sample after a time t of immersion (mg) and m_f is the final weight of film sample after incubation and drying (g). This relation takes into account the loss of material that may occur during immersion.

Contact Angle Measurements. Contact angles were measured at room temperature using goniometer (Digidrop, GBX, France) equipped with a CCD camera (25 frames/s) and the GBX software (Windrop, GBX, France) on films previously dried over silica gel. Five microliter of each tested FSL [water and ethanol 95% (v/v)] were deposited on PHBV films. Contact angles were determined by first angle of contact between the liquid and the PHBV film surface. Measurements were done in triplicate.

Mechanical Properties. Stress–strain measurements were performed on dog-bone shaped specimens (4 mm wide) die-cut from both hot-pressed sheets (0.25 mm thick) and injection-molded trays using a tensile testing machine (Zwick BZ2.5/TN1S, France). Ten specimens of each sample were tested at a cross head speed of 10 mm min⁻¹ and a initial gage length of 40 mm. PHBV films after the contact with food simulants were previously wiped with a tissue paper to remove the excess of food liquid simulant on the film surface. Nominal stress at break (σ_b), nominal elongation at break (ϵ_b), and Young's modulus (E) were determined from stress–strain curves. Films were stored in desiccators at 53% RH and 20°C over a saturated salt solution of MgNO₃ before analysis.

Water Vapor Permeability. Water vapor permeability of films (mol m⁻¹ s⁻¹ Pa⁻¹) was gravimetrically determined at 23°C using a modified ASTM procedure. Samples were hermetically sealed (with Teflon seals) in a glass permeation cell containing distilled water. The permeation cell stocking water was placed at 23°C in a desiccator containing silicagel, thus obtaining a RH gradient equal to 0–100%. The water vapor transfer through the exposed film area (9.1 cm²) was measured from the cell weight loss as a function of time. Cells were weighed using a four-digit balance every 24 h over a 10 days period, until steady-state vapor flow had been reached. At least three samples of each

type of film were tested and water vapor permeability (WVP) was calculated from the following equation:

$$\text{WVP} = \frac{w \times x}{A \times P} \quad (2)$$

where w is the slope of the weight loss versus time, (mol s^{-1}), Δx is the film thickness (m), A is the area of exposed film (m^2), and Δp is the water vapor pressure differential across the film (at 23°C , $\Delta p = 2.808 \times 10^3$ Pa, assuming that the RH on the silicagel is negligible).

Overall Migration (OM). Overall migration (expressed in mg per dm^2 of food contact surface) was evaluated according to the European Standard EN 1186 series. Six food liquid simulants (FSLs) were used: water, acetic acid 3% (w/v), ethanol 20% (v/v), olive oil as well as the alternative fatty food simulants ethanol 95% (v/v) and iso-octane. For each FSL, overall migration tests were carried out by total immersion of 0.5 dm^2 of material in 50 mL of FSL in a closed glass bottle and storage at 40°C for 10 days. Experiments were realized in triplicate.

Scanning Electron Microscopy (SEM). SEM observations of both the top view surfaces and cryo-fractured cross-sections of films were performed to evaluate changes in film morphology (presence or not of holes). Micrographs were performed using an emission scanning electron microscope (SEM S-4500, Hitachi, Japan) operating with 5 keV of beam and a detector for secondary electrons. The films were mounted and coated with gold/palladium on an ion sputter coater.

Differential Scanning Calorimetry (DSC). Crystallization and melting temperatures of PHBV materials before and after the contact with FSLs were investigated using differential scanning calorimetry (Q200 modulated DSC, TA Instruments, New Castle, DE). Before analysis, materials (around 10 mg) were stored at 53% RH and 20°C over a saturated salt solution of MgNO_3 . After equilibration, samples were immediately placed in open aluminum pans (Tzero aluminum hermetic, TA Instruments New Castle, DE) and hermetically sealed. Measurements were done with a thermo-modulated calorimeter. Samples were first heated from -40 to 190°C at a heating rate of $50^\circ\text{C min}^{-1}$ and maintained for 10 min at 190°C to erase the thermal history, then cooled down to -40°C at a rate of $10^\circ\text{C min}^{-1}$ and finally heated again until 190°C at a rate of $10^\circ\text{C min}^{-1}$, under a nitrogen flux of 50 mL min^{-1} . The melting (T_m) and crystallization (T_c) temperatures were determined as the peak temperatures of melting endotherm and crystalline exotherm, respectively. The degree of crystallinity achieved during crystallization, X , was calculated using the following equation:

$$X = \frac{\Delta H_m}{\Delta H_0} \quad (3)$$

where ΔH_m is the melting enthalpy of PHBV samples (J g^{-1}) and ΔH_0 is the melting enthalpy of a perfect crystal of PHBV with 0% of valerate: 146 J g^{-1} .^{15,16}

Thermogravimetric Analysis (TGA). Thermogravimetric analysis was performed using a TGA Q50 device from TA Instruments. The films samples were heated with ramp of $10^\circ\text{C min}^{-1}$ from room temperature to 700°C under nitrogen flow

Table I. Weight Uptake of PHBV Films after Immersion in Different FSLs and Contact Angle Values of Water and Ethanol 95% (v/v) on PHBV Films

FSL	FSL sorption ($\text{mg g}^{-1} \text{ d.b.}^{\text{a}}$)	Contact angle ($^\circ$)
Water	7.3 ± 0.2	90.3 ± 6.5
Acetic acid 3% (w/v)	9.7 ± 0.2	^b
Ethanol 20% (v/v)	3.0 ± 0.3	^b
Ethanol 95% (v/v)	38.1 ± 1.2	31.5 ± 1.2
Isooctane	1.6 ± 0.6	^b

^a Weight of FSL sorbed by 1 g of dry film after the contact with food simulants, thus taking into account the weight loss of PHBV.

^b Tests were not realized.

(60 mL min^{-1}). Approximately 10 mg of sample was heated in an open platinum crucible. The analysis consisted in registering the weight loss of the sample as a function of temperature (TA analysis software). Temperature of degradation (T_d) was determined at the peak of the weight derivative curve, i.e. at the highest rate of weight loss. The degradation percentage was calculated from the difference weight of the sample at $T_{d \text{ onset}}$ and at $T_{d \text{ offset}}$ divided by the initial sample weight.

Molar Mass. The molar mass of PHBV was evaluated by gel permeation chromatography (GPC) with a differential refractive index detector (Waters 410) and a 600 mm column PL gel MixC $5 \mu\text{m}$. Samples were prepared by dissolving 25 mg of PHBV-based material in 5 mL of chloroform, in a closed tube, under stirring, at 50°C . Then, samples were filtered on Macherey-Nagel CHROMAFIL syringe filter (O-20/15 MS type), with pores' size of $0.2 \mu\text{m}$. Chloroform was used as eluent at 1 mL min^{-1} and 20 μL of sample solution were injected. The GPC equipment was calibrated using polystyrene narrow standards (Agilent Standards as the reference polymer, peak molecular weight range from 162 to $1,238,000 \text{ g mol}^{-1}$).

RESULTS AND DISCUSSION

Physical–Chemical Stability of PHBV Films Under Food

Contact Conditions

The physical–chemical stability of PHBV (3% of valerate) films under food contact conditions was investigated by using food simulating liquids (FSL) to mimic the contact with different types of food, i.e. aqueous, fatty, alcoholic, and/or acid while conducting experiments at the conditions set out by the European Plastics Regulation,² i.e., 10 days of immersion at 40°C . It was first assessed in terms of affinity of PHBV films towards the different selected FSLs, by both weight uptake and contact angle measurements. Then, the impact of food contact on the evolution of the mechanical properties, water vapor permeability and the overall migration of PHBV films was assessed, these latter properties being among the necessary keys to insure food quality and safety to consumers.

Weight Uptake of PHBV Films After Contact with FSLs

Values of weight uptake of PHBV films after immersion during 10 days at 40°C in all FSLs are summarized in Table I. Very low weight uptake values were obtained for water, acetic acid 3%

Table II. Tensile Properties and Water Vapor Permeability (WVP) of PHBV Films Before (Control) and after Immersion in Different Food Simulant Liquids (FSL)

FSL	Elongation at break (%)	Stress at break (MPa)	Young's modulus (GPa)	WVP ($\times 10^{-13}$ mol mm^{-2} s^{-1} Pa^{-1})
Control	2.8 \pm 0.3	29.0 \pm 2.0	2.5 \pm 0.2	5.8 \pm 1.5
Water	2.3 \pm 0.3	33.2 \pm 3.5	2.5 \pm 0.1	8.2 \pm 2.2
Acetic acid 3% (w/v)	2.5 \pm 0.2	27.6 \pm 1.7	2.6 \pm 0.1	^a
Ethanol 20% (v/v)	2.8 \pm 0.3	26.9 \pm 1.9	2.4 \pm 0.2	^a
Ethanol 95% (v/v)	3.6 \pm 0.1	22.6 \pm 1.4	1.7 \pm 0.1	9.9 \pm 3.1
Isooctane	2.4 \pm 0.1	28.4 \pm 2.2	2.7 \pm 0.0	^a

^a Tests were not realized.

(w/v), ethanol 20% (v/v) and iso-octane [from 1.6 mg g⁻¹ d.b. for iso-octane up to 9.7 mg g⁻¹ d.b. for acetic acid 3% (w/v)], whereas a great weight uptake was noticed in the specific case of ethanol 95% (v/v) (38.1 \pm 1.2 mg g⁻¹ d.b.). The same effect was already observed for PET, with a material swelling induced only in the case of contact with ethanol 95% (v/v) without any or only minor modification with others FSLs such as acetic acid 3% (w/v) and ethanol 10% (v/v).¹³ For PHBV, it is worth noting that such weight uptake was not accompanied with a measurable swelling phenomenon since no significant change in the film thickness was noticed. These results suggested a very high affinity of PHBV towards ethanol 95% (v/v) as compared to other tested FSLs. This was further confirmed by contact angle measurements, since a much more lower contact angle value was obtained for a drop of ethanol 95% (v/v) deposited on the surface of a PHBV films (31.45 \pm 1.2°) than for a drop of water (90.3 \pm 6.5°) (Table I). Such a sensibility towards ethanol 95% (v/v) was already reported for other polyesters, especially PET and PLA^{13,14} and suggested that the sorption and diffusion of ethanol 95% (v/v) within PHBV films would affect the structure of the polymer, by potentially inducing macroscopic defects, molecular rearrangement due to increasing the segmental mobility and even chain degradation, and thus impacting functional properties such as mechanical properties and water vapor permeability.

Mechanical Properties of PHBV Films After Contact with FSLs

Tensile tests were performed to evaluate the impact of contact with FSLs during 10 days at 40°C on the mechanical properties of PHBV films (Table II). The mechanical properties of PHBV films were stable over 10 days of contact with all FSLs [water, acetic acid 3% (w/v), ethanol 20% (v/v) and iso-octane], except with ethanol 95% (v/v) where a plasticizing effect was noted (Table II). This was reflected by a significant decrease in the stress at break (from 29 to 22.6 MPa) and the Young's modulus (from 2.5 to 1.7 GPa), together with an increase in the elongation at break (from 2.8 to 3.6%), without significant change in the energy at break. The improved flexibility of PHBV films after contact with ethanol 95% (v/v) could be directly attributed to the sorption of solvent previously observed, that could distinctively result in a decrease in the glass transition temperature, a decrease in the molecular weight ascribed to a degradation of

the polymer, and/or a decrease in the crystallinity degree of the polymer matrix. Such a plasticizing effect was also reported in the case of PET films,¹³ whereas PLA films were described to become more fragile after the contact with ethanol 50% (v/v).¹⁴ This highlights that the stability of polyesters towards food is not unanimous and should be studied case by case.

Water Vapor Permeability of PHBV Films After Contact with FSLs

The evolution of water vapor permeability of PHBV films was investigated for contact with water and ethanol 95% (v/v) only (Table II). WVP was not significantly changed after contact with water, but slightly increased after contact with ethanol 95% (v/v). This result is coherent with the previous plasticizing effect of ethanol 95% (v/v) on PHBV, which would favor the mobility of polymer chains and thus the diffusion of water vapor molecules. On the other hand according to the free volume theory, the possible degradation of polyester chains because of the sorption of ethanol could result in an increase of amorphous area and consequently facilitate the diffusion of low molecular weight molecules and water vapor.

Overall Migration Tests

The European Plastics Regulation (EU) No 10/2011 adopts rules on plastic materials and articles intended to come into contact with food, which are based on synthetic polymers and on synthetic or natural polymers that have been chemically modified. It also explicitly covers bio-based and biodegradable plastics based on polymers manufactured by microbial fermentation. PHBV polymers (Poly(3-D-hydroxybutyrate-co-3-D-hydroxyvalerate) (CAS: 80181-31-3) are authorized by the Plastics Regulation, but are subjected to specific specifications (e.g., regarding the average molecular weight and purity requirements) as set out in Annex I of the Plastics Regulation. Overall migration tests were performed to evaluate the compliance of the produced PHBV films with the overall migration limit and their suitability to be used as food contact materials (Figure 1). For this purpose the overall migration was measured into food simulants, which represent the hydrophilic, amphiphilic, and lipophilic properties of food and therefore, the chemical characteristics that lead to a transfer of substances from the food contact material into the food. Migration in any of the used simulants [water, acetic acid 3% (w/v) and ethanol 20%

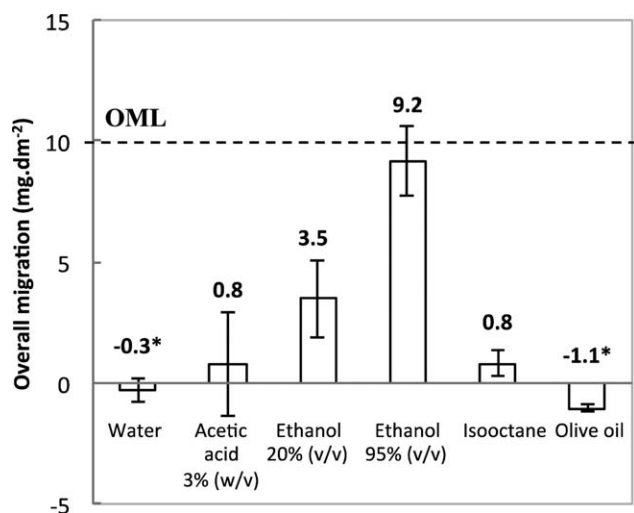


Figure 1. Overall migration of PHBV in food simulant liquids (water, acetic acid 3% (w/v), ethanol 20% (v/v), ethanol 95% (v/v), iso-octane, and olive oil). OML is the overall migration limit (10 mg dm⁻² of film surface area) set out by the Commission Regulation (EU) N° 10/2011.² *The negative value is within the analytical tolerance of the method. No overall migration was detectable.

(v/v), iso-octane and olive oil] was below the overall migration limit of 10 mg of total constituents released per dm² of food contact surface (10 mg dm⁻²) under the standardised testing conditions of 10 days at 40°C (set out in Annex V of the Plastics Regulation).

This means that tested PHBV film is in compliance with the overall migration limit set out in the European Plastics Regulation (EU) No 10/2011 for any long term storage at room temperature or below, including heating up to 70°C for up to 2 h, or heating up to 100°C for up to 15 min.

Generally, the overall migration is the parameter linked to the inertness of a (plastic) material. The European Framework Regulation (EC) No 1935/2004 on food contact materials sets out, that food contact materials shall not release their constituents into food in concentrations that could change the composition of the food. A release of 10 mg of constituents per 1 dm² surface area of plastic food contact material is established as the limit (overall migration limit) above that the migration is regarded as an unacceptable change of the food.

The overall migration experiments show that the tested PHBV films is sufficiently inert and stable in contact with all simulants, either highly polar [water, acetic acid 3% (w/v) and ethanol 20% (v/v)] or apolar (iso-octane and olive oil). These results are coherent with already published values for iso-octane and ethanol 10% (v/v).^{9,11} However, it is worth noting that a very high (nearly excessive) migration was noted in the case of ethanol 95% (v/v), with an overall migration value of 9.2 mg dm⁻². PHBV was strongly affected by contact with ethanol 95% (v/v). These results are completely coherent with the respective sensitivity of PHBV films towards FSLs and the effects previously described on mechanical properties and water vapor permeability. Ethanol 95% (v/v) is commonly considered as the

worst case in terms of chemical stability for other polyesters, especially PET and PLA, for which similar overall migration results were obtained.^{13,14,17} Furthermore, results highlighted that iso-octane could be considered as an appropriate alternative to vegetable oil for simulating the contact with fatty food-stuffs in the case of PHBV overall migration testing since similar overall migration values were obtained in both cases.

To conclude on this part, it should be reminded that two concomitant phenomena are governing food/packaging interactions and chemical migration. The first important parameter is the solubility of migrants in the respective FSL. For instance, if a migrant has a poor solubility in the food simulant, it will tend to be retained by the polymer rather than to migrate into the simulant.^{14,18} This is often the case with nonpolar migrants in nonpolar polymers in contact with very polar food simulants, such as water, 3% acetic acid or ethanol 20% (v/v). The second phenomenon is based on the affinity between FSLs and the polymer, which is responsible for the possible absorption of FSLs by the polymer matrix that can lead to increase the diffusivity of migrants in the polymer matrix. This phenomena has been extensively observed on a large panel of polymers including PLA, PET, PVC, and PP for which solvent or FSL sorption dramatically promoted chemical migration.^{12,19–23} Additionally, the sorption of FSL could induce a plasticizing effect, as observed in the present study for ethanol 95% (v/v), but also changes in the crystalline structure and/or the degradation of the polymer. On the basis of what was published for PET and PLA, different assumptions could be here proposed to explain higher overall migration values obtained with ethanol 95% (v/v). Among these, (1) a decrease of the glass transition temperature of polymer amorphous phases, (2) a decrease of polymer crystallinity, and/or (3) the hydrolysis of the polyester inducing the formation of low molecular weight substances can be considered as the most likely ways favoring an enhanced mobility of both polymer chains and migrants.^{13,24} All these assumptions will be validated in the following part by studying the evolution of the structural stability of PHBV films in contact with FSLs, which will be evaluated at different scales (macroscopic to molecular).

Structural Stability of PHBV Films Upon Food Contact

The structural stability of PHBV films in contact with FSLs was evaluated at different scales, from macroscopic to molecular levels. Morphological changes were first evaluated at the macroscopic scale by SEM observations of the appearance of films. At the micro/nanometric scale, attention was given to the impact of contact with food simulants on the degree of crystallinity, which was evaluated by differential scanning calorimetry (DSC). Finally, the structural stability was evaluated at the molecular scale by focusing on the thermal transition (melting and crystallization) temperatures (evaluated by DSC), the thermal degradation temperature (evaluated by TGA), and the molecular weight of polymer chains (evaluated by GPC).

SEM Observations

SEM observations of both the surface and the cross-section of PHBV films were carried out to investigate eventual changes in the macroscopic morphology of materials (Figure 2). White

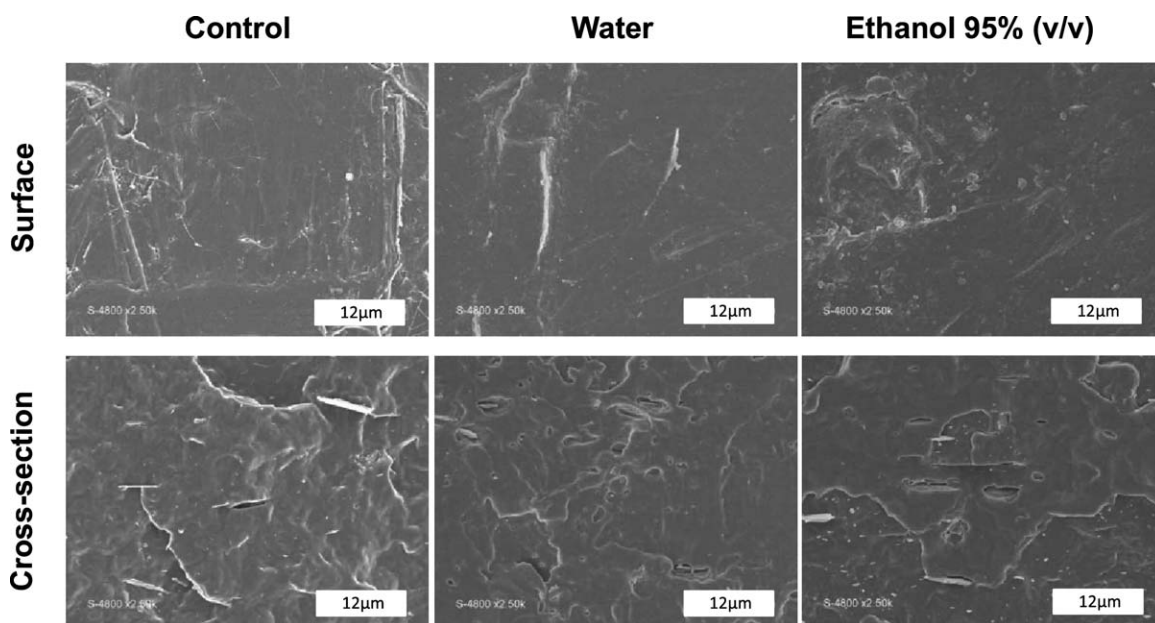


Figure 2. SEM observations of the surface and cryo-fractured cross-section of PHBV films before and after the incubation in food simulant liquids at 40°C for 10 days.

flakes of several micrometers were observed on all pictures of the cross-section of materials and were attributed to crystals of boron nitride used as nucleating agents.²⁵ No obvious differences were noticed after contact with all FSLs, as illustrated here for water and ethanol 95% (v/v).

Structural Characteristics of PHBV Films after Contact with FSLs

The impact of contact with FSL was investigated through some structural (degree of crystallinity and polymer molecular weight) and thermal properties (crystallization, melting, and thermal degradation temperatures) of PHBV films and results are summarized in Table III.

Generally, no structural change was noticed for iso-octane, even after contact at 40°C for 10 days. This confirms the excellent stability of PHBV films in contact with very apolar solvents such as iso-octane (log P = 4.5). As regards the other FSLs [water, acetic acid 3% (w/v), ethanol 20% (v/v), and ethanol

95% (v/v)], structural parameters evolved following the same tendency.

The degree of crystallinity was decreased after contact with water, acetic acid 3% (w/v) ethanol 20% (v/v), and ethanol 95% (v/v), meaning that the amount of amorphous phase increased, in favor of an improved extensibility of PHBV after the immersion. This decrease in crystallinity may be related to the decrease in the molecular weight of polymer chains, as measured by GPC, and was also revealed by the decrease in the melting temperature (T_m) (Table III). To corroborate this hypothesis, FT-IR analysis was performed on PHBV samples before and after contact with all the FSL (Data not shown). Discriminant analysis ($n = 6$) did not reveal any significant difference of the spectral fingerprint due to detectable modifications of chemical structure of polymer suggesting a slight extent of polymer modification during the contact. The polymer degradation could be ascribed to the hydrolysis reactions induced by the presence of water molecules and alcohol groups, which have

Table III. Crystallinity Degree, Molar Mass (in Weight and Polydispersity index) and Thermal Properties of PHBV Films Before (Control) and after incubation in Different FSLs During 10 days at 40°C

Samples	Crystallinity (%)	Molar mass (M_w) (g mol ⁻¹) ^a	Molar mass (I_p)	T_m (°C)	T_c (°C)	$T_{c\ onset} - T_c$ (°C)	T_{deg} (°C)
Control	64.9 ± 1.0	219 × 340	2.1	173.0 ± 0.3	122.8 ± 0.1	9.3 ± 0.6	285 ± 8
Water	61.3 ± 1.1	194 × 300	2.2	170.9 ± 0.1	118.8 ± 0.6	6.3 ± 1.2	281 ± 1
Acetic acid 3% (w/v)	63.0 ± 0.6	-	-	169.4 ± 0.3	121.4 ± 0.0	8.9 ± 0.5	282 ± 2
Ethanol 20% (v/v)	61.9 ± 0.3	-	-	171.9 ± 0.5	120.6 ± 1.5	9.4 ± 0.1	282 ± 1
Ethanol 95% (v/v)	59.5 ± 0.4	199 × 700	2.2	168.6 ± 0.4	118.2 ± 0.2	7.3 ± 1.3	286 ± 1
Iso-octane	63.5 ± 0.3	215 × 600	2.1	172.8 ± 0.5	121.7 ± 0.3	8.8 ± 0.3	286 ± 2

^aTypical error of 1–2%.

been proven to favor PHBV's chain scission.²⁶ These degradation reactions were already highlighted for other polyesters such as PLA¹⁴ and PET²⁷ in the case of contact with ethanol-based simulants. Such effect was all the more pronounced for water and ethanol 95% (v/v). Depending on the location of the chain cleavage, the hydrolysis reaction can lead to the formation of new breakdown components of low mass molecular weight, which may migrate from the PHBV towards food.

The crystallization temperature (T_c) was also significantly decreased after contact with water and ethanol 95% (v/v), highlighting a hindered crystallization process, which was coherent with previous results, i.e. the decrease in the M_w of polymer chains. The parameter $T_{c(\text{onset})} - T_c$ was a measure of the overall crystallization rate, this value decreasing from $9.3 \pm 0.6^\circ\text{C}$ for the original PHBV down to respectively $6.3 \pm 1.2^\circ\text{C}$ and $7.3 \pm 1.3^\circ\text{C}$, for PHBV films after contact with water and ethanol 95% (v/v). This revealed that the crystallization rate of PHBV after FSL contact was higher than that of original one, which was also in agreement with previous results.

It was shown that such structural changes induced by the contact with FSLs had no significant impact on the thermal stability of PHBV films since no significant change of the temperature of thermal degradation (T_{deg}) was noticed (Table III).

To conclude, the occurrence of structural changes was related to the affinity of PHBV with the different tested FSLs. As expected, the most relevant structural changes (decrease in M_w and crystallinity) were noticed for films in contact with ethanol 95%, supporting the plasticizing effect, the increase in the water vapor permeability and the large overall migration value. However, it must be pointed out that similar structural changes were also observed in the case of contact with water, whereas no significant impact on physical–chemical stability and overall migration was noticed. This highlights that such a study of the structural stability of PHBV films upon food contact (by using food simulants) is very useful to understand changes in physical–chemical properties, but not enough to fully predict the physical–chemical stability and the chemical safety of PHBV. Indeed, as previously mentioned, the solubility of additives and potential breakdown components in FSLs (that is supposed to be low in water) is another important parameter to be taken into account for the assessment.

CONCLUSION

It can be concluded from the overall migration tests that the tested PHBV films can be used as food contact materials for all types of food, i.e. high water activity, acidity, alcohol, or fatty foods. It was shown that iso-octane could be considered as an appropriate substitute for fatty simulants (vegetable oil) for PHBV overall migration testing whereas ethanol 95% (v/v) induces an excessive overall migration value. The functional properties of PHBV films (mechanical properties and water vapor permeability) were very stable after contact at 40°C during 10 days with all FSLs tested (water, acetic acid 3% (w/v), ethanol 20% (v/v), iso-octane and olive oil), except with ethanol 95% (v/v), corroborating the possibility to use PHBV films as

packaging material for a wide range of foodstuff. As previously evidenced for other polyesters such as PET, ethanol 95% (v/v) was clearly identified as the most severe food simulant for PHBV films, with a strong impact on their physical–chemical and chemical stability. In this case, a high sorption value together with a significant plasticizing effect and an increase in the water vapor permeability were noticed, which was mainly explained by a decrease in both the molecular weight and the crystallinity degree of PHBV films.

Globally, a strong dependence between the structural, physical–chemical and chemical stability of polymers was demonstrated. However, it was shown that the occurrence of structural changes, intimately related to the affinity between the polymer and the FSL, was not the unique parameter governing the physical–chemical stability and the inertness of PHBV films. Indeed, in the case of water, a decrease in the polymer molecular weight and crystallinity were observed with no impact on functional properties and overall migration, meaning that such structural changes do not fully predict the physical–chemical stability and chemical safety of PHBV films. Thus, an in-depth interest should be also given to the affinity between potential migrants and FSLs and their migration behavior.

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