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Inclusion et libération de molécules antioxydantes dans un emballage à base d'Acide Poly Lactique en contact alimentaire

Majid Jamshidian

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Je dédie ce travail

A ma chère femme qui a toujours cru en moi

A ma chère petite fille que j'aime

A ma famille entière

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*In theory, there is no difference
between theory and practice.*

But in practice, there is.

Jean, L.A. van de Snepscheut

Glossaire

AFM	Atomic force microscopy
AP	Ascorbyl palmitate
ASME	American society of mechanical engineers
ASTM	American society for testing and materials
AT	α -tocopherol
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
D	Diffusion coefficient
DSC	Differential scanning calorimetry
EVOH	Ethylene vinyl alcohol
FTIR	Fourier transform infrared spectroscopy
GPPS	General purpose poly(styrene)
HDPE	High density poly(ethylene)
HIPS	High impact poly(styrene)
HPLC	High pressure liquid chromatography
K	Partition coefficient
LDPE	Low-density poly(ethylene)
LLDPE	Linear-low-density poly(ethylene)
MMT	Montmorillonite
MWCNT	Multiwalled-carbon nanotube
OMMT	Organo-montmorillonite
OPLA	Oriented poly(lactic acid)
OPP	Oriented poly(propylene)
OPS	Oriented poly(styrene)
PBAT	Poly(butylene adipate terephthalate)
PBS	Poly(butylenes succinate)
PBST	Poly(butylene succinate terephthalate)
PC	Poly(carbonate)
PCL	Poly(ϵ -caprolactone)

PEA	Poly(ester amide)
PEG	Poly(ethylene glycol)
PEGMA	Poly(ethylene-glycidyl methacrylate)
PEO	Poly(ethylene oxide)
PET	Poly(ethylene terephthalate)
PET	Poly(ethylene terephthalate)
PG	Propyl gallate
PGA	Poly(glutamic acid)
PHB	Poly(3-hydroxybutyrate)
PHV	Poly(hydroxyl valerate)
PLA	Poly(lactic acid)
PDLA	Poly(D-lactic acid)
PLLA	Poly(L-lactic acid)
PP	Poly(propylene)
PS	Poly(styrene)
PTMAT	Poly(tetramethylene adipate terephthalate)
PVA	Poly(vinyl alcohol)
PVC	Poly(vinyl chloride)
QSPR	Quantitative structure property relationships
SAXS	Small-angle X-ray scattering
TBBQ	2- <i>tert</i> -butyl-1,4-benzoquinone
TBHQ	Tert-butylhydroquinone
TEM	Transmission electron microscopy
TPS	Thermoplastic starch
WAXS	Wide range X-ray scattering
XPS	X-ray photoelectron spectroscopy
XRD	X-ray Diffraction

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I . Introduction et objectifs de l'étude

1- Introduction et objectifs de l'étude

Les emballages passifs ont quatre fonctions principales, qui sont : la sécurité des aliments, la protection, la praticité et la communication tandis que les emballages actifs offrent une protection active libérant en permanence des composés actifs tels que des antioxydants, des agents antimicrobiens, des enzymes, des arômes et des produits nutraceutiques. Les emballages actifs par leur concept innovant et en raison de leurs interactions avec l'emballage, l'aliment et l'environnement, génèrent de plus longues durées de conservation des aliments, une protection plus élevée des saveurs, ainsi que de faibles teneurs en additifs et conservateurs dans des formulations alimentaires tout en préservant la qualité du produit.

Les produits d'oxydation de faible poids moléculaire donnent un mauvais goût, détruisent les éléments nutritifs essentiels, et produisent des composés toxiques. Dans les graisses et les huiles, les antioxydants retardent le début d'oxydation ou ralentissent les réactions d'oxydation. Le but principal est d'utiliser un antioxydant comme un additif alimentaire pour maintenir la qualité de l'aliment et de prolonger sa durée de conservation plutôt que d'améliorer la qualité des aliments. Afin de protéger l'oxydation des lipides, les antioxydants sont traditionnellement ajoutés dans la formulation alimentaire initiale, ou bien les aliments sont trempés dans des solutions contenant des antioxydants. L'ajout direct d'antioxydants dans les aliments est limité par leur participation à des réactions complexes devenant parfois pro-oxydantes et le manque de sélectivité pour cibler la surface des aliments où la plupart des réactions d'oxydation se produisent.

Les emballages contenant l'antioxydant sont une catégorie importante des emballages actifs qui permet d'augmenter la durée de stockage des aliments. Dans la littérature, différents antioxydants ont été incorporés dans plusieurs films de polymère et leurs migrations dans les aliments réels et les aliments simulants ont été étudiés. Dans certains cas, les effets de la migration des antioxydants sur l'oxydation des lipides et sur l'odeur ont également été étudiés, mais, dans la plupart des études, les objectifs principaux ont été les taux, les effets et les mécanismes de la libération des antioxydants.

Les propriétés mécaniques, physico-chimiques et barrières affectent les propriétés techniques et la stabilité du polymère dans des conditions différentes. Les propriétés structurales peuvent influencer le taux de migration des antioxydants. Puisque, les effets des agents actifs sur les

propriétés des polymères dans lesquels ils ont été ajoutés n'avaient pas été réellement traités auparavant, nous les avons étudiés par une étude de caractérisations multi-échelles de polymères avant et après l'ajout d'antioxydants. Nous avons également développé les emballages actifs par deux modes d'élaboration.

Les antioxydants utilisés dans cette étude sont autorisés par la réglementation alimentaire européenne et ont essentiellement deux effets : ils évitent la dégradation du polymère au cours du processus d'extrusion et ils modifient les propriétés du polymère. D'autre part ils peuvent contrôler le phénomène d'oxydation des lipides alimentaires. Comme la migration des additifs de polymères en contact avec des produits alimentaires n'est généralement pas nulle, nous pouvons utiliser les composés qui sont habituellement consommés dans les aliments comme les antioxydants naturels ou synthétiques autorisés dans les emballages. Dans ce cas, si ces agents migrent vers les produits alimentaires, les préoccupations concernant la libération d'agents non-autorisés ne se posent pas et les composés libérés peuvent agir comme agents de conservation. L'addition d'antioxydants synthétiques dans les formulations de polymères est une pratique de plus en plus courante en tant que moyen de protection de la dégradation des polymères pendant leur traitement et pendant leur durée de vie. Les antioxydants protègent le polymère contre la formation de produits d'oxydation et de composés indésirables qui peuvent migrer vers les aliments provoquant une diminution de la qualité des produits. La migration potentielle de ces substances néoformées dans le produit emballé est une question qui devient essentielle du point de vue de la qualité et de la sécurité alimentaire.

Nous avons choisi l'acide Poly lactique (PLA) comme matrice porteuse d'antioxydants. Le PLA est un polyester aliphatique biodégradable fabriqué à partir de ressources renouvelables comme le maïs et les betteraves à sucre après fermentation de l'amidon et condensation de l'acide lactique. Le PLA offre de grandes possibilités dans une large gamme d'applications de produits et rivalise avec le polystyrène dans les nombreuses applications d'emballage alimentaire. Le PLA est un matériau transparent et rigide avec un taux de cristallisation relativement faible qui en fait un candidat prometteur pour la fabrication de films bi-orientés, de récipients thermoformés et de bouteilles.

Après un Etat de l'Art concernant le PLA (production, caractéristique physico-chimiques, transfert moléculaire), nous avons étudié l'influence des antioxydants naturels et synthétiques

incorporés dans le PLA sur différentes propriétés du film. Les molécules sélectionnées ont été deux antioxydants naturels (α -tocopherol et ascorbyl palmitate), et quatre antioxydants de synthèse (butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ)). Les films de PLA ont été obtenus par casting et par extrusion. Les propriétés mécaniques et thermiques ont été analysées à l'aide d'une machine universelle d'essai et d'un calorimètre différentiel. Les propriétés barrières à l'oxygène et à la vapeur d'eau ont été évaluées. Des techniques précises comme l'AFM, les spectroscopies XPS, Raman et FTIR, la calorimétrie différentielle ainsi que la microscopie électronique à transmission ont été appliquées pour étudier l'incorporation des antioxydants dans la matrice du PLA. Nous avons observé que le mode d'élaboration du PLA et le pourcentage d'antioxydants incorporés jouent un rôle primordial sur les propriétés du matériau. Une étude multi-échelle a été réalisée pour chaque concentration d'antioxydant ainsi que pour chaque mode d'élaboration du PLA. Le phénomène de transfert moléculaire (diffusion et partage) a été étudié pour chaque antioxydant à partir du film vers les différents aliments simulés à deux températures (20 et 40°C). Pour valider les résultats expérimentaux, une relation entre la structure et les propriétés a été développée avec un coefficient de régression entre prévisions et mesure de 0,97.

Introduction and objectives of the study

Passive packaging has four main functions including food safety, protection, convenience, and communication while active packaging provides active protection by continuously releasing active compounds such as antioxidants, antimicrobials, enzymes, flavors and nutraceuticals to package content. Active packaging as an innovative concept and due to its interaction with the package, the food and the environment, generates longer shelf-life foods, higher protection of flavors and nutrients and lower use of additives and preservatives in food formulations to maintain product quality.

Oxidation produces low-molecular-weight off-flavor compounds, destroys essential nutrients, and produces toxic compounds and dimmers or polymers of lipids and proteins. In fats and oils, antioxidants delay the onset of oxidation or slow the rate of oxidizing reactions. The main purpose of using an antioxidant as a food additive is to maintain the quality of that food and to extend its shelf life rather than improving the quality of the food. To protect food lipid oxidation, antioxidants traditionally are mixed into initial food formulations, or alternatively foods are dipped into solutions containing antioxidants. Limitations of direct addition of antioxidants to foods include specific limit of activity due to their participation in complex reactions in food systems, and lack of selectivity to target the food surface where most oxidative reactions occur.

Antioxidant-active packaging is an important category of active packaging and promising technique for extending food shelf life, different antioxidants have been impregnated in several polymer films and their migrations in real foods and food simulants have been studied. In some cases, effects of migrated antioxidants on lipid oxidation and odor stability have also been investigated, but in most studies important objectives were rates, effects, and mechanisms of antioxidants' release. Mechanical, physicochemical and barrier properties affect technical properties and stability of polymer in various conditions and structural properties can influence the rate and amount of active agents whom desired to migrate. As the effects of added active agents on polymer properties had not been well discussed before, we decided to include a complete characterization (multi-scales) study of polymer properties influenced by antioxidants addition. We also tried to get a better understanding of

antioxidants mode of incorporation into polymer matrix and investigate their potential interactions with polymer functional groups.

We chose food antioxidant because of their dual actions; firstly, for their release providing a continuous control of food lipid oxidation, and secondly for their influence to avoid polymer degradation during extrusion process and probable modification effect on polymer properties. Addition of synthetic antioxidants in polymer formulations is a common practice as a way to protect polymeric degradation during processing and also during their life-time. Antioxidants protect the polymer against the formation of undesirable oxidative and low molecular compounds which can migrate to foods causing a decrease in food quality. However, the potential migration of these substances to the packaged product has been an issue that should be considered to maintain food quality and safety.

As migration of additives from polymers in contact with foodstuffs usually exists, we used antioxidant compounds which are usually consumed in foods. In this case if antioxidants migrate to food, the safety concerns regarding to release of unpermitted agents do not arise and released compounds can act as preservatives released from active packaging.

Poly lactic acid (PLA) was chosen as the polymeric matrix carrying the antioxidants. PLA is a biodegradable aliphatic polyester made from 100% renewable resources, such as corn and sugar beets following fermentation of starch and condensation of lactic acid. PLA offers great promise in a wide range of commodity applications and competes with polystyrene for many food packaging applications. It is a highly transparent and rigid material with a relatively low crystallization rate that makes it a promising candidate for the production of biaxially oriented films, thermoformed containers and stretch-blown bottles.

In first chapter of this study the PLA production, characteristics, modifications method, release studies and etc, are described as a review article.

Then, at the first step of our research study, we investigated the precise influences of added antioxidants on PLA properties at macro, micro and nano scales. Two natural antioxidants including α -tocopherol and ascorbyl palmitate and four synthetic antioxidants including butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) were incorporated into PLA by two methods of solvent-casting and extrusion. Mechanical and thermal properties were analyzed using a universal

testing machine and a differential scanning calorimetry, respectively. Oxygen and water vapor permeability barrier properties were tested. The highly precise techniques such as atomic force Microscopy (AFM), X-ray photoelectron spectroscopy (XPS), Raman spectroscopy, Fourier transform infrared spectroscopy (FTIR), DSC and transmission electron microscopy (TEM) were applied to investigate the antioxidants incorporation in PLA matrix.

At the second step of research study, the release behavior of applied antioxidants from solvent-cast and extruded on PLA into food simulating liquids including 95%, 50% and 10% ethanol at 20°C and 40°C was accomplished. The diffusion coefficient (D) and partition coefficient (K) of each antioxidant were calculated.

Finally, a QSPR model based on D coefficients was developed with an accuracy of 97%.

Publications

1. **Jamshidian, M.**, Tehrany, E. A., Imran, M., Jacquot, M., & Desobry, S. (2010). Poly-Lactic Acid: production, applications, nanocomposites, and release studies. *Comprehensive Reviews in Food Science and Food Safety*, 9(5), 552-571.
2. **Jamshidian, M.**, Tehrany, E. A., Cleymand, F., Leconte, S., Falher, T., & Desobry, S. (2012). Effects of synthetic phenolic antioxidants on physical, structural, mechanical and barrier properties of poly lactic acid film. *Carbohydrate Polymers*, 87, 1763-1773 .
3. **Jamshidian, M.**, Tehrany, E. A., Cleymand, F., Imran, M., Akhtar, M.J & Desobry, S. (2012). Structural, mechanical and barrier properties of active PLA-antioxidant films. *Journal of Food Engineering*, 110, 380-389.
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10. Imran M., Revol-Junelles A.M., René N., **Jamshidian M.**, Akhtar M.J., Tehrany E.A., Jacquot M., Desobry S. (2012). Microstructure and physico-chemical evaluation of nanoemulsion-based antimicrobial peptides embedded in packaging films, *Food hydrocolloids* (Acceptée).

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13. Imran M., Revol-Junelles A.M., Francius G., **Jamshidian M.**, & Desobry S. (2011). Diffusion kinetics of fluorescently labeled pore-forming lantibiotic from nanocarriers and pre-loaded nano-active biopolymer, *ACS Nano* (Soumise).

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1. **Jamshidian, M.**, Tehrany, E. A., & Desobry, S. (2012). PLA-antioxidant active packaging; a healthier option for protecting oils and oily foods. 103rd AOCS Annual Meeting & Expo. California, USA. April 29 - May 2, 2012.
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3. **Jamshidian, M.**, Tehrany, E. A., & Desobry, S. (2011). Poly lactic acid active packaging: migration of natural and synthetic antioxidants. European polymer congress epf2011, Granada, Spain, June 26 - July 1, 2011.

II . Synthèse bibliographique

Acide poly lactique: Production, Applications, Nanocomposites, et Etudes de Relargage

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Résumé

Les défis environnementaux, économiques et de sécurité des aliments ont conduit les scientifiques de l'emballage et les producteurs à remplacer partiellement les polymères issus de la pétrochimie par d'autres biodégradables. L'objectif général de cette revue est de présenter l'acide polylactique (PLA), un thermoplastique compostable et biodégradable fabriqué à partir de ressources renouvelables. Les propriétés du PLA et ses modifications via différentes méthodes, comme l'utilisation des modificateurs de réseau, les mélanges, la copolymérisation, et les traitements physiques sont mentionnés; ces modifications sont rarement discutées ensemble dans les autres états de l'art critiques. Les méthodes de transformation industrielle pour la production de différents films du PLA, simples ou multicouches ainsi que les contenants (bouteilles et gobelets) sont présentés. Les capacités du PLA pour être un matériau pour emballage actif fort dans différents domaines nécessitant des caractéristiques antimicrobiennes et antioxydant sont discutées. Par conséquent, les applications des nanomatériaux en combinaison avec le PLA pour la création de nouveaux nanocomposites plus performants sont également couvertes. Ces approches peuvent modifier les faiblesses du PLA dans certaines applications d'emballage alimentaire. Les approches de la nanotechnologie sont appliquées à la science des aliments, et en particulier à la science des matériaux d'emballage ayant des performances élevées pour de faibles concentrations et prix. La nano-recherche est révolutionnaire dans la science des emballages alimentaires. La conjugaison d'un biomatériau à 100% et de nanoparticules ouvre de nouvelles possibilités pour remplacer les polymères d'origine pétrochimique et, accessoirement, répondre aux préoccupations environnementales et de santé qui seront de plus en plus importantes dans l'avenir.

Poly-Lactic Acid: Production, Applications, Nanocomposites, and Release Studies

Majid Jamshidian, Elmira Arab Tehrani, Muhammad Imran, Muriel Jacquot, and Stéphane Desobry

Abstract: Environmental, economic, and safety challenges have provoked packaging scientists and producers to partially substitute petrochemical-based polymers with biodegradable ones. The general purpose of this review is to introduce poly-lactic acid (PLA), a compostable, biodegradable thermoplastic made from renewable sources. PLA properties and modifications via different methods, like using modifiers, blending, copolymerizing, and physical treatments, are mentioned; these are rarely discussed together in other reviews. Industrial processing methods for producing different PLA films, wrappings, laminates, containers (bottles and cups), are presented. The capabilities of PLA for being a strong active packaging material in different areas requiring antimicrobial and antioxidant characteristics are discussed. Consequently, applications of nanomaterials in combination with PLA structures for creating new PLA nanocomposites with greater abilities are also covered. These approaches may modify PLA weaknesses for some food packaging applications. Nanotechnology approaches are being broadened in food science, especially in packaging material science with high performances and low concentrations and prices, so this category of nano-research is estimated to be revolutionary in food packaging science in the near future. The linkage of a 100% bio-originated material and nanomaterials opens new windows for becoming independent, primarily, of petrochemical-based polymers and, secondarily, for answering environmental and health concerns will undoubtedly be growing with time.

Introduction

Today, polymers and materials used for food packaging consist of a variety of petrochemical-based polymers, metals, glass, paper, and board, or combinations hereof. The durability and degradability of packaging materials are 2 contradictory subjects; the 1st is desirable for packaging stability and protection for its contents during shelf life and the 2nd for its rapid degradation in the environment (Bohlanan 2005).

Advantages of petrochemical-based polymers, which encouraged industries to use them are: (a) low cost and high-speed production; (b) high mechanical performance; (c) good barrier properties; and (d) good heat sealability. On the other hand, several disadvantages include: (a) declining oil and gas resources; (b) increasing oil and gas prices during recent decades; (c) environmental concerns for their degradation or incineration and global warming; (d) uneconomical costs and cross-contaminations in their recycling; and (e) consumer toxicity risks about their monomers or oligomers migrating to edible materials (Amass and others 1998; Chandra and Rustgi 1998; Mohanty and others 2000; Siracusa and others 2008).

Mechanical recycling (segregated plastics, mixed plastics), biological recycling (sewage, compost, soil), and energy recovery (incineration, pyrolysis) are 3 alternative ways for plastics waste management, with each having some advantages and disadvantages as to economical, processing, and technological aspects (Scott 2000).

The above-mentioned concerns are negligible for biopolymers concerning the biodegradation process that takes place in nature. Biodegradation is defined as the degradation of a polymer in natural environments that includes changes in chemical structure, loss of mechanical and structural properties, and finally, changing into other compounds like water, carbon dioxide, minerals, and intermediate products like biomass and humic materials. The natural environments contain chemical, biological, and physical forces with impinging factors like temperature, humidity, pH, O₂ presence, and so on, which determine the rate and products of the biodegradation process (Zee 2005).

Biopolymers are produced from natural resources and crude oil. Four categories of biopolymers are recognized: (a) extracted directly from natural raw materials, such as polysaccharides like starch and cellulose; proteins like gelatin, casein, and silk; and marine prokaryotes; (b) produced by chemical synthesis from bio-derived monomers such as poly-lactic acid (PLA), also known as poly(lactic acid) in the literature; (c) produced by microorganisms or genetically modified bacteria such as polyhydroxyalkanoates (PHA), polyhydroxybutyrate (PHB), hydroxyl-valerate (PHV), bacterial cellulose, xanthan, and pullan; and (d) produced from crude oil like aliphatic and aromatic polyesters, polyvinyl alcohol, and modified

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polyolefins, which are sensitive to temperature and light (Chandra and Rustgi 1998; Clarinval and Halleux 2005).

PLA or poly-lactide was discovered in 1932 by Carothers (at DuPont). He was only able to produce a low molecular weight PLA by heating lactic acid under vacuum while removing the condensed water. The problem at that time was to increase the molecular weight of the products; and, finally, by ring-opening polymerization of the lactide, high-molecular weight PLA was synthesized. PLA was 1st used in combination with polyglycolic acid (PGA) as suture material and sold under the name Vicryl in the U.S.A. in 1974 (Mehta and others 2005).

In comparison to other biopolymers, the production of PLA has numerous advantages including: (a) production of the lactide monomer from lactic acid, which is produced by fermentation of a renewable agricultural source corn; (b) fixation of significant quantities of carbon dioxide via corn (maize) production by the corn plant; (c) significant energy savings; (d) the ability to recycle back to lactic acid by hydrolysis or alcoholysis; (e) the capability of producing hybrid paper-plastic packaging that is compostable; (f) reduction of landfill volumes; (g) improvement of the agricultural economy; and (h) the all-important ability to tailor physical properties through material modifications (Dorgan and others 2000).

Briefly, PLA is based on agricultural (crop growing), biological (fermentation), and chemical (polymerization) sciences and technologies. It is classified as generally recognized as safe (GRAS) by the United State Food and Drug Administration (FDA) and is safe for all food packaging applications (Conn and others 1995; FDA 2002).

Production steps, general properties, applications, processing technologies, modifications, and biodegradability of PLA are presented in this review. Consequently, migration and release studies of active compounds and PLA abilities making it a potential active food packaging are also discussed; finally, recent different types of nanocomposites used for improving PLA applications are reviewed.

PLA Production

Lactic acid (2-hydroxy propionic acid), the single monomer of PLA, is produced via fermentation or chemical synthesis. Its 2 optically active configurations, the L(+) and D(-) stereoisomers are produced by bacterial (homofermentative and heterofermentative) fermentation of carbohydrates. Industrial lactic acid production utilizes the lactic fermentation process rather than synthesis because the synthetic routes have many major limitations, including limited capacity due to the dependency on a by-product of another process, inability to only make the desirable L-lactic acid stereoisomer, and high manufacturing costs (Datta and Henry 2006).

The homofermentative method is preferably used for industrial production because its pathways lead to greater yields of lactic acid and to lower levels of by-products. The general process consists of using species of the *Lactobacillus* genus such as *Lactobacillus delbrueckii*, *L. amylophilus*, *L. bulgaricus*, and *L. leichmanii*, a pH range of 5.4 to 6.4, a temperature range of 38 to 42 °C, and a low oxygen concentration. Generally, pure L-lactic acid is used for PLA production (Mehta and others 2005).

PLA has a variable molecular weight and only its high molecular weight polymer is used in the packaging industry. Three ways are possible for the polymerization of lactic acid; (a) direct condensation polymerization; (b) direct polycondensation in an azeotropic solution (an azeotrope is a mixture of 2 or more chemical liquids in such a ratio that its composition cannot be changed by simple distillation. This occurs because, when an azeotrope is boiled, the

resulting vapor has the same ratio of constituents as the original mixture); and (c) polymerization through lactide formation. The 1st method is based on esterification of monomers by the aid of some solvents and exudated water is removed using progressive vacuum and high temperatures. Obtaining high molecular weight polyesters with good mechanical properties via this method is not easy, although precondensates may be of interest for the preparation of biodegradable glues or lacquers, since the -OH and -COOH end groups allow cross-linking with suitable inorganic or organic multivalent additives (Hartmann 1998).

Producing high molecular weight PLA polymers by direct polycondensation in an azeotropic solution and also application of some catalysts is more practicable. The azeotropic solution helps to decrease the distillation pressures and facilitates PLA separation from the solvent by application of molecular sieves. The variety and content of catalysts, solvent volume percentages, and the reaction time on the preparation of PLA have been studied. The results identified by using improved experimental equipment, the proper complex catalyst, and solvent volume ratio, in order to obtain a molecular weight of PLA of 6.6×10^4 (Li and others 2006).

Polymerization through lactide formation is being industrially accomplished for high molecular weight PLA production. Lactide is a cyclic dimer formed by removing water under mild conditions and without solvent. L-lactide, meso (L,D) lactide, and D-lactide are products of L-lactic acid and D-lactic acid. The terms poly lactide and poly (L-lactide) have been used in many references instead of PLA.

Lactide purification is accomplished by vacuum-distillation of high temperatures. After the vacuum-distillation of L-lactide, high molecular weight PLA with a controlled optical and crystal purity is formed by ring-opening polymerization. Ring-opening polymerization of lactide can be carried out in melt or solution by cationic, anionic, and coordination mechanisms, depending on the initiator utilized. The most considered active initiator for the L-lactide ring-opening polymerization is stannous octoate (bis 2-ethyl hexanoate, SnOct₂), which causes a low degree of racemization at high temperature. It has a low toxicity and is accepted by FDA (Puaux and others 2007).

A kinetics study for ring-opening polymerization of L-lactide with stannous octoate has been done and a correlated mathematical modeling developed for that (Mehta and others 2007).

The choice of initiator system, co-initiator as chain control agent, catalyst concentration, monomer-to-initiator ratio, and polymerization temperature and time significantly affect the polymer properties. Properties such as molecular weight, degree of crystallinity, and residual monomer content, in turn affect the physico-mechanical properties of polylactide and its copolymers (Vink and others 2004).

Figure 1 shows PLA production steps by ring-opening polymerization using stannous octoate as an initiator.

New ideas for decreasing PLA final price and making production processes more eco-friendly, in comparison to earlier production process, include usage of crop residue (stems, straw, husks, and leaves) from corn or, potentially, other crops, and use of unfermentable residues as a heat source, as well as substituting some part of electricity energy by wind power energy. These approaches decrease the consumption of fossil fuels and corn starch as raw materials and also diminish polluting air, water, and waste emissions to the environment (Vink and others 2003).

One of the most positive points of PLA production in comparison with the other hydrocarbon-based polymers is the decrease of CO₂ emission. Carbon dioxide is believed to be the most

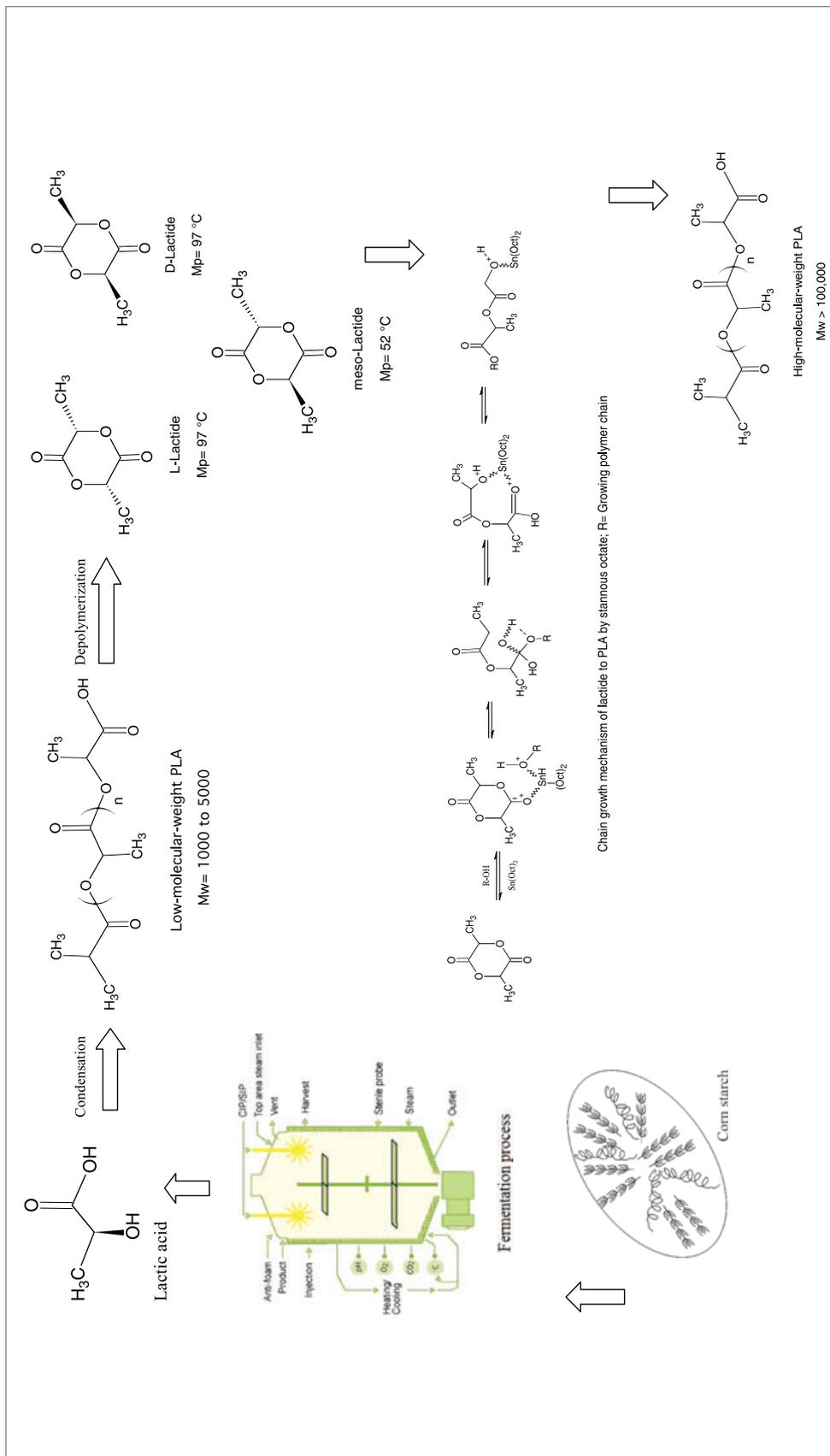


Figure 1—Current production steps for PLA.

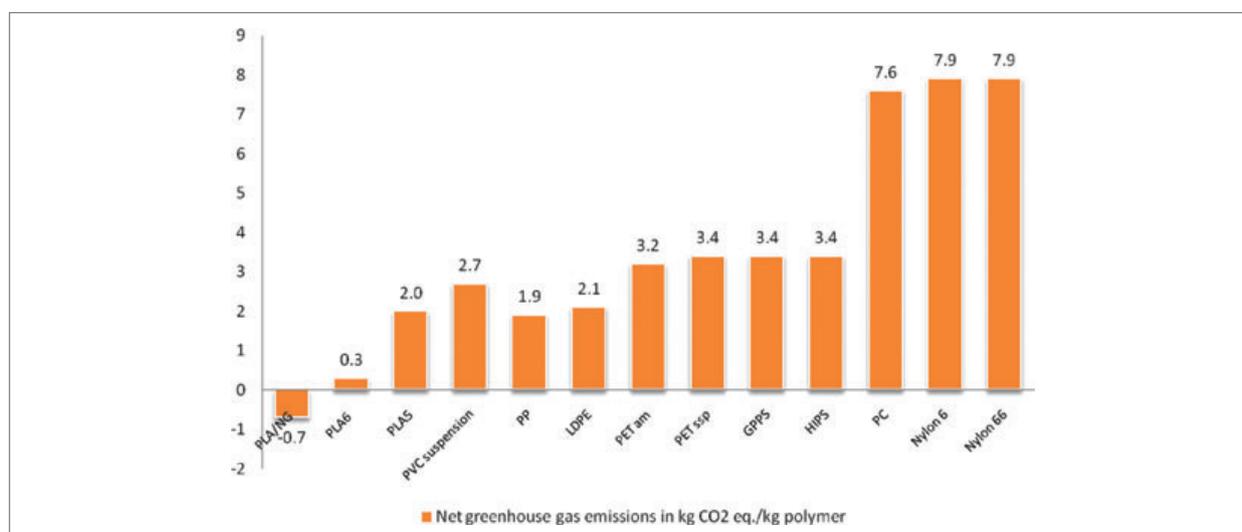


Figure 2—Net greenhouse gas emission of commercial PLAs and other polymers. PLA/NG = NatureWorks® PLA next generation, PLA5 = NatureWorks® PLA in 2005, PLA6 = NatureWorks® in 2006, HIPS = high impact poly(styrene), PC = poly(carbonate), GPPS = general purpose poly(styrene), PET am = PET amorph, PET ssp = PET solid sate polycondensed.

important contributor to global climate change and its warming. Because, carbon dioxide is absorbed from air when corn is grown, use of PLA has the potential to emit fewer greenhouse gases compared to competitive hydrocarbon-based polymers. “Net” or “residual” emissions are calculated as total emissions from the cradle to the factory gate minus carbon dioxide uptake that occurs during corn production. This amount is negative for present PLA production. It means the total CO₂ consumption from the cradle to factory is more than its emission to the environment (Bogaert and Coszach 2000). Vink and others (2003) concluded if all produced PLA articles enter into composting process which emits CO₂ in atmosphere; Nevertheless, their net CO₂ emission is less than for other polymers. Vink and others (2007) showed the net greenhouse gas emissions of NatureWorks® PLA polymers decreased from 2 kg CO₂ eq./kg polymer in 2003 to 0.3 kg in 2006. The authors also estimated the -0.7 kg of CO₂ for next PLA generation using wind energy in the near future. By this opportunity, PLA can even become a greenhouse gas sink by the implementation of a new process technology combined with the use of green power to drive the production processes (Figure 2).

NatureWorks® LLC, the present leader in PLA technology, has a 50 to 50 joint venture between Cargill incorporated and Dow Chemical Co. and was formed in November 1997. In 2002, they started the world’s first full-scale PLA plant in Blair, Nebraska, U.S.A., capable of producing 140,000 metric tons per year. NatureWorks® entered into a joint venture between Cargill and Teijin Limited of Japan in December 2007 (www.natureworkslc.com).

Other major companies involved in PLA manufacturing are Toyobo, Dai Nippon Printing Co., Mitsui Chemicals, Shimadzu, NEC, Toyota (Japan), PURAC Biomaterials, Hycail (The Netherlands), Galactic (Belgium), Cereplast (U.S.A.), FkuR, Biomer, Stanelco, Inventa-Fischer (Germany), and Snamprogetti (China) (Wolf 2005; Platt 2006).

Sources for lactic acid fermentation

NatureWorks® exclusively uses corn starch as raw material for lactic acid production via lactic fermentation. Many studies have been conducted to find other sources of carbohydrates for lac-

tic acid production. The use of a specific carbohydrate feedstock depends on its price, availability, and purity. Some agricultural by-products, which are potential substrates for lactic acid production include, cassava starch, lignocellulose/hemicellulose hydrolysates, cottonseed hulls, Jerusalem artichokes, corn cobs, corn stalks, beet molasses, wheat bran, rye flour, sweet sorghum, sugarcane press mud, cassava, barley starch, cellulose, carrot processing waste, molasses spent wash, corn fiber hydrolysates, and potato starch (Reddy and others 2008).

Other sources of carbohydrate for lactic acid production include kitchen wastes (Kim and others 2003; Zhang and others 2008), fish meal wastes (Huang and others 2008), and paper sludge (Budhavaram and Fan 2007). By using kitchen wastes, concerns about waste management in crowded cities could be automatically eased. Additionally, some parts of carbohydrates from wastes will return to the production cycle of lactic acid and, as a result, decrease a large amount of corn consumption. By using other carbohydrate sources rather than corn, the criticisms and debates about utilizing a food source as packaging material will be defused (Zhang and others 2008).

PLA Processing Technologies for Food Applications

The methods of manufacture for biopolymers are all established polymer-manufacturing techniques, but the control and application of these methods must be varied to cope with certain factors associated with exploiting the advantages of biopolymers. The manufacturing routes all show certain fundamental similarities, with the major differences depending on whether a thermoset or thermoplastic biopolymer is to be processed.

The conditions in biopolymer processes such as injection molding are least damaging to polymer melts, and most problematic in continuous processes like extrusion, particularly in processes where the extrudate is stretched, such as film blowing. The limiting factors for processing conditions for biopolymers are the same as for petrochemical-based ones: degradation at the upper limits of temperature and shear, and lack of homogeneity at the lower limits. However, these limits are somewhat more tightly drawn at the upper limits for biopolymers. The results of exceeding these upper limits are degradation of the polymer, resulting in molding

Table 1—Processing possibilities of typical commercial biodegradable polymers (Clarival 2002; Clarival and Halleux 2005, NatureWorks® datasheets).

	Injection molding	Extrusion	Extrusion blow molding	Cast film extrusion	Blow molding	Fiber spinning	Thermoforming
Starch	×	×	×	×			
Cellulose	×	×			×		
PHB	×	×	×	×	×		×
PHB-PHV	×	×	×	×	×	×	×
PLA	×	×		×	×	×	×
PBS	×	×					×
PCL	×	×	×		×	×	×
PBST	×	×		×			×
PBAT		×	×	×			
PTMAT		×	×	×		×	
PVA	×	×		×		×	×
PPPE + additives	×	×	×	×	×	×	×
Starch + PVA	×	×		×	×	×	
Starch + cellulose acetate	×	×	×		×		×

PHB = Poly(3-hydroxybutyrate); PHV = Poly(hydroxyl valerate); PBS = Poly(butylenes succinate); PCL = Poly(ϵ -caprolactone); PBST = Poly(butylene succinate terephthalate); PBAT = Poly(butylene adipate terephthalate); PTMAT = Poly(tetramethylene adipate terephthalate); PVA = Poly(vinyl alcohol).

defects such as weld lines, discoloration, or a strong odor in the final product (Johnson and others 2003).

Processing possibilities of typical commercial biodegradable polymers are presented in Table 1.

Commercial PLA resins are packaged in crystalline and amorphous pellet forms. Crystalline and amorphous pellets look significantly different. Semicrystalline pellets are opaque and amorphous pellets are transparent. Different types of PLA resins with different application ranges are being produced and each customer should specify packaging demands and match them with PLA data sheets. In Table 2, available commercial PLA resins for food packaging applications are characterized.

The processing technologies for producing different packaging applications with PLA resins are mentioned here.

Drying

As PLA is sensitive to high-relative humidity and temperature conditions, and for minimizing the risk of its molecular degradation, it is necessary to be dried less than 0.01% w/w. This value is expressed as 0.025% w/w or below in NatureWorks® data sheets. PLA resins normally are packaged with moisture content below 0.04% w/w in moisture-resistant foil liners to maintain that moisture level, and so the drying process is essential. Drying conditions are dependent on temperature, time, air flow rate, and dew point. Amorphous pellets must be dried below the T_g (43 to 55 °C) to prevent the resin pellets from sticking together, which can bridge and plug the dryer (Lim and others 2008).

For crystalline types, the recommended temperatures and times range between 80 to 100 °C and 4 to 2 h, respectively. Typical drying conditions are 4 h at 80 °C (175 °F) or to a dew point of -40 °C (-40 °F), with an airflow rate greater than 0.032 m³/min per kg (0.5 cfm/lb) of resin throughout (NatureWorks® PLA processing guide for biaxially oriented film 2005b). PLA is a hygroscopic thermoplastic and readily absorbs moisture from the atmosphere; so its resins should not be exposed to atmospheric

Table 2—Commercial Ingeo™ PLA resins adapted by NatureWorks® data sheets.

Product code	Applications	Usages
2002D	Extrusion, thermoforming	Dairy containers, food serviceware, transparent food containers, blister packaging, cold drink cups
3001D	Injection molding for applications with heat deflection temperatures lower than 55 °C (130 °F)	Cutlery, cups, plates, and saucers, and outdoor novelties
3051D		
3251D	Injection molding, having higher melt flow capability than other PLA resins for easier molding of thin-walled parts	Injection molding applications, both clear and opaque, requiring high gloss, UV resistance, and stiffness
4032D	Biaxially oriented films with use temperatures up to 150 °C (300 °F), barrier to flavor and grease, and oil resistance	Laminations, printed films with higher curing temperatures, other packaging applications
4042D	Biaxially oriented films with use temperatures up to 130 °C (265 °F), barrier to flavor and grease, and superior oil resistance	Candy twist-wrap, salad, and vegetable bags, window envelope film, lidding film, label film, other packaging applications
4060D	Heat sealant with a seal initiation temperature of 80 °C	Can be coextruded with other PLA resin to form a sealant layer for biaxially-oriented PLA film
7000D	Injection stretch blow molding, for 1:2 stage operations	Fresh dairy, edible oils, fresh water
7032D	Injection stretch blow molding, for 1:2 stage operation	Fruit juices, sports drinks, jams, and jellies

conditions after drying and the packages should be kept sealed until ready to use and promptly be dried and resealed if not entirely used.

Extrusion

The 1st major step in the conversion of plastic resin into films, sheets, containers and so on, is to change the pellets from solid to liquid or molten phase in an extruder.

Extrusion is a common way for melting thermoplasts and it is the 1st step for extrusion coating, cast film extrusion, blown film extrusion, and other polymer processes.

Screw extruders are typically used in the polymer industry. They consist of an electrically heated metal barrel, a hopper for feeding the resin, a motor for rotating a screw, and a die where the polymer melt exists. So, the combination of thermal energy generated by a heater and frictional heat due to friction between the plastic and the screw and barrel provide sufficient heat to melt the pellets.

The L/D ratio, which is the ratio of flight length of the screw to its outer diameter, determines the shear and residence time of the melt. Screws with a large L/D ratio provide greater shear heating, better mixing, and longer melt residence time in the extruder. Another important screw parameter is the compression ratio, which is the ratio of the flight depth in the feed section to the flight depth in the metering section. The greater the compression ratio a screw possesses, the greater the shear heating it provides (Giles and others 2005).

Recommended extrusion conditions for PLA pellets include general purpose screws with L/D ratios from 24:1 to 30:1 and compression ratio of 2.5:1 to 3:1, melt temperature of 200 to 220 °C, and also smooth barrels (NatureWorks® PLA 4042 data sheet 2006a).

Injection molding

Injection molding involves melting a thermoplastic by extrusion, injecting the polymer melt into a mold, cooling the part, and finally ejecting the part. Most polymers can be injection molded so long as they can flow and fill the mold cavity easily. The commonly used polymers are Poly(ethylene terephthalate) PET, Poly(styrene) (PS), Poly(propylene) (PP), high-density poly(ethylene) (HDPE), Low-density poly(ethylene) (LDPE), nylon, and Poly(vinyl chloride) PVC.

An injection molding machine is similar to an extruder and the main difference between 2 machines is in their screw operation. In an extruder, the screw rotates continuously providing output of continuous and long product (pipe, rod, sheet), but the screw of an injection molding machine, which is called reciprocating screw, does not only rotate but also moves forward and backward according to the steps of the molding cycle. The mold is equipped with a cooling system providing controlled cooling and solidification of the material. Injection molding may be used to manufacture a wide variety of parts such as bottle caps, food trays, containers, and preforms for blow molding (Rosato and others 2000).

Injection mold-grade PLA is injection molded on most conventional equipment, but there could be some torque limitations if the screw design has a high compression ratio. Compression ratios of 2.5 to 3 should be adequate and the recommended melting temperature is 200 to 205 °C. Since PLA has a lower glass transition temperature (about 58 °C) than PS or PET, it might take a little longer time to set up in the mold (NatureWorks® PLA injection molding guide for 3051D 2006b).

Physical aging occurs when a polymer is in a nonequilibrium state and is caused by molecular relaxations that are biased in the direction required to drive the material closer to equilibrium. This phenomenon is very common and is encountered in thermoplastics moldings that have been cooled rapidly from an elevated temperature during the shaping operation such as injection molding process (White 2006). Physical aging significantly affects the physical properties of the amorphous phase in glassy or partly glassy polymers. The effect of aging takes place around T_g and can be noticed by shrinkage of specific volume, decreases in specific enthalpy and entropy, and a decrease in molecular mobility. These effects are associated with decrease of free volume, which controls the mobility of large segments of the polymer chains and affects the mechanical properties of polymers such as shrinkage, stiffness, brittleness, and decrease in damping (Ke and Sun 2003b; Acioli-Moura and Sun 2008).

Injected molded PLA articles are relatively brittle, which caused by rapid physical aging of polymer since ambient temperature is approximately 25 °C below the T_g . Cai and others (1996) studied physical aging behavior of PLA in different times and temperatures by DSC. The results confirmed the augmentation of endothermic peak at T_g by increasing the aging time, which is related to the increase of the excess enthalpy of relaxation. The rate of physical aging was very fast initially and decreased as time increased. They also showed that the aging temperature of 37 °C had the maximum enthalpy of relaxation, but by increasing the temperature above 60 °C the enthalpy of relaxation was greatly reduced. However, storage conditions for injected molded PLA articles that are especially intended for further processing (like preforms) should be carefully controlled.

Shear-controlled orientation in injection molding (SCORIM) is a nonconventional injection molding technique that allows for the enhancement of the mechanical properties of semicrystalline polymers and has additional degrees of freedom over conven-

tional injection molding. SCORIM technique manipulates the structure development of a solidifying polymer melt through an in-mold shearing action, thereby tailoring the morphology and hence controls the mechanical properties of polymers (Grossman 1995). Ghosh and others (2008) investigated the effect of operative parameters of SCORIM on Poly(L-lactic acid) (PLLA). They showed some modifications in energy at break and maximum stress of all the SCORIM processed PLLA. The overall increments in maximum stress and energy at break were 134% and 641%, respectively.

Blow molding

Blow molding is a process of blowing up a hot thermoplastic tube (called parison or preform, a term derived from the glass industry) with compressed air to conform to the shape of a chilled mold and releasing the finished product from the mold. The most widely used materials for blow molding are LDPE, HDPE, PP, PVC, and PET (Lee and others 2008).

There are 3 common types of blow molding: extrusion blow molding, injection blow molding, and injection stretch blow molding (ISBM).

Extrusion blow molding. Extrusion blow molding begins with extruding a polymer melt into a parison. The chilled mold is then closed, followed by blowing air through a blow pin to inflate the parison to conform to the shape to the mold cavity. After cooling the plastic, the mold is opened, and the part is ejected. Containers produced by extrusion blow molding must meet minimum stiffness requirements to undergo filling on automated lines. They must avoid, or limit, unsightly bulging under weight of their contents, both alone or when stacked. They must also withstand normal impacts of handling, transport, and accidental dropping. Such impact must be absorbed by the container walls, weld lines (pinch-off and handle areas), and screw cap closure threads, often under extremes of temperature. Basic polymers for extrusion blow molding are HDPE, PP, and PVC. These polymers are sometimes coextruded with Ethylene vinyl alcohol (EVOH) or nylon to provide a better gas barrier (Lee 2006).

PLA containers are not produced by this method, because of a lack of the required physical and mechanical properties; and PLA resins for this application have not been yet produced.

Injection blow molding. Injection blow molding is a 2-step process for making plastic containers. This method produces a molded parison called a preform. This method is preferred over extrusion blow molding for making small parts that require high-production volumes and closer quality dimensions. Injection blow molding consists of injecting a thermoplastic material into a cavity and around a core rod producing a hollow test tube like shape (preform). The molded preform still on the core rod is transferred to the blow mold. The mold is clamped around the preform and air is blown into it to shape to the cavity. The preform is injected onto a support pin or core, which forms a neck with threads to their required dimensions. The preform is then blown against the cavity wall to its final shape (Lee 2006; Lee and others 2008).

The use of preform allows the manufacture of bottles with more precise detail in the neck and finish (threaded) area than extrusion blow molding.

Injection blow molding requires lower degree of melt strength than extrusion blow molding and its tooling costs are higher. Common polymers for this method are PS, LDPE, Linear low-density poly(ethylene) (LLDPE), HDPE, PP, PVC, and PET (Lee and others 2008).

This process is typically limited to the production of relatively small bottles and PLA pellets are rarely recommended to be processed by this method.

Injection stretch blow molding. Injection stretch blow molding (ISBM) is an extension of injection blow molding with 2 modifications: (a) the preform is significantly shorter than the bottle and (b) a stretch rod is used to stretch the preform in the axial direction. This process became known in the blow molding industry with the introduction of plastic or PET soft drink bottles.

While all blow molding processes involve blowing air to stretch the parison or preform in some fashion, ISBM is designed to achieve and retain biaxial orientation to significantly improve gas barrier properties, impact strength, transparency, surface gloss, and stiffness. Biaxial orientation is achieved by elongating the preform with the stretch rod and blowing air to stretch the preform in a direction perpendicular to the axis of the preform, while precisely controlling a temperature warm enough to allow rapid inflation and molecular orientation, but cool enough to retard relaxation of its molecular structure once oriented (Rosato and others 2000).

PET bottles for carbonated soft drinks are the most common food packaging applications of this process. The combination of stretching by rod and blowing air at high pressure (about 4 MPa) induces biaxial molecular orientation, thereby making the bottles a better barrier to carbon dioxide and stronger to withstand the internal pressure (Rosato and others 2000; Lee and others 2008).

ISBM-grade PLA resins are accessible and they are generally used for bottles for different foods like fresh dairy liquids, fruit juices, sport drinks, edible oils, and so on.

ISBM-grade PLA resin is typically run at lower processing temperatures than bottle-grade PET and the blow molding conditions include: preform temperature at 80 to 100 °C, stretch rod speed 0.8 to 1.2 m/s, and blow mold temperature at 100 to 120 °C. In fact, the heating of the preforms is critical in getting a container with good clarity and material distribution. Normal preform temperatures for running on a 2-step process have been between 80 and 100 °C. This temperature may be lower or higher depending on the preform design, bottle design, and reheating equipment that is being used (NatureWorks® PLA ISBM bottle guide 2005a).

Preform design is critical in getting a container with good clarity and physical properties. Designing a preform for use as a PLA container is specific to the blow mold equipment, bottle design, and mold tooling.

Cast film extrusion

The cast film process involves extruding a molten polymer through a slit die and drawing it around 2 or more highly polished high-speed rolls, typically chrome-plated and water-cooled. In less than 1 revolution, the chill roll solidifies the product as it draws it down to the correct thickness. Cast film is used in packaging, food wrap, substrate for coating, protective film, agricultural film for weed control, general purpose polyethylene film as a protective barrier to prevent scratching of parts during shipment, and many other applications (Giles and others 2005).

Due to rapid cooling by the chilled rolls, cast films typically have a low degree of crystallinity and transparent appearance. Besides providing good optical properties, cast film extrusion has the advantages of high production rate, good control of film thickness and uniformity, and little or no additive is required for processing.

Similar to PP, PET, and PS films, the physical properties of PLA films can be enhanced through orientation. Uniaxial orientation of PLA is achieved with conventional machine direction orientation (MDO) rolls. Since PLA tends to neck in (neck in happens

by contacting the melt film with the 1st point of the die; the hot film shrinks on its way down so its width from the die to the chill roll is reduced. At the same time, beading or thickening of the edges occurs) during drawing, nipped rolls are usually required. It is possible to improve both the thermal resistance and impact resistance of PLA films or sheets by drawing, orientation, and crystallization to the same level of strength and stiffness as oriented polypropylene (OPP) or PET, while maintaining its high transparency. An oriented film is obtained by stretching it to 2 to 10 times its original length at 60 to 80 °C, and further annealing it at temperatures between the stretching temperature and melting point. An oriented film may be either processed for dry lamination, printing, and heat seal or other applications including various types of packaging (Kawashima and others 2002).

Thermoforming

Thermoforming is a generic term encompassing many techniques for producing useful plastic articles from flat sheets. Thermoforming is a process that deals with the pressing or drawing of pliable plastic into final shape by vacuum or air pressure. A wide range of thermoplastics, including PP, LDPE, LLDPE, HDPE, PET, PS, and nylon, may be thermoformed. Food packaging is the largest application for thermoformed containers, trays, cups, and tubs. Typical thermoforming steps are clamping, heating, shaping, cooling, and trimming (Throne 1996).

PLA sheet can be thermoformed with vacuum, compressed air/vacuum, or only compressed air assistance. The radiant heater of the thermoforming line for PLA must be adjusted to very low temperatures. Preheating is not absolutely necessary; however it has the general advantage that the sheet is homogeneously preheated.

PLA sheet is quite brittle at room temperature and requires some special handling and storage considerations. There is a greater risk of cracking and breaking during transporting compared with Oriented poly(Styrene) (OPS) or PET. Neither the sheet nor the finished product can be stored at temperatures above 40 °C or greater than 50% relative humidity. These conditions minimize moisture uptake and consequently sheet blocking, and resistance to unwinding. Exposure to high temperatures or humidity, even for a short period, can cause the material to deform and eventually break down. Sheet and formed products must be transported in cooled trucks and stored in a climate-controlled warehouse. The toughness of PLA increases with orientation, and therefore, thermoformed articles are less brittle than PLA sheet, particularly in the regions that have been highly stretched during the forming operations rather than flanges and lips. So, flange or lip areas that receive less orientation tend to be more brittle than the rest of the thermoformed part (NatureWorks® processing guide for thermoforming articles 2005c; Patey 2010).

PLA is frequently thermoformed using forming ovens, molds, and trim tools designed for PET or polystyrene. Because of higher shrinkage of PP than PLA, the molds and trim tools designed for PP are less optimally used for PLA.

PLA has a lower softening temperature than PET or PS. Typically oven settings are about 55 °C (100 °F) or lower than PS and about 40 °C (75 °F) or lower than PET oven settings. The sheet should be about 90 to 110 °C (190 to 230 °F) entering the mold. Aluminum molds are recommended for thermoforming PLA. PLA thermal properties indicate that the cooling time in the mold will be greater for PLA than either PS or PET (NatureWorks® processing guide for thermoforming articles 2005c).

Patey (2010) discussed some essential factors for PLA thermoforming process. According to his suggestions, optimizing a

conventional polymer thermoforming line for PLA just needs some minor modifications in tools and equipments.

PLA crystallinity diminishes its shrinkage after thermoforming process. Uradnisheck (2009) showed the shrinkage of PLA thermoformed articles is minimized by longer dwell times and crystallinity of polymer. He concluded that the crystallinity in a thermoformed article dwelling in the heated mold raised to a higher level due to supplemental crystallinity generated in the preheat step and forming or stretching step.

PLA Thermal Stability

PLA is thermally unstable and exhibits rapid loss of molecular weight as the result of thermal treatment at processing temperatures. The ester linkages of PLA tend to degrade during thermal processing or under hydrolytic conditions. PLA undergoes thermal degradation at temperatures lower than the melting point of the polymer, but the degradation rate rapidly increases above the melting point. It has been postulated that thermal degradation mainly occurs by random main-chain scissions. Several reactions such as hydrolysis, depolymerization, oxidative degradation, and inter- and intramolecular trans-esterification reactions to monomer and oligomeric esters, are suggested to be involved in the degradation process during thermal treatments (Taubner and Shishoo 2001; Södergård and Stolt 2002).

Taubner and Shishoo (2001) studied 3 parameters on thermal degradation of PLA during extrusion processing, including processing temperature (210 and 240 °C), residence time in the melt (1.75 and 7 min), and the inherent moisture content of polymer. Their results confirmed higher polymer degradation by increasing processing temperature and time. In a temperature of 210 °C, the loss in M_n (Number-average molecular weights) was less dependent on the residence time in the melt compared to when processed at a temperature of 240 °C. The presence of moisture in the material affected the loss in M_n to a great extent when processing was done at 210 °C. The rate of degradation at 240 °C and 7 min was so high that they concluded the moisture content in the polymer probably does not contribute further to the degradation process.

Different factors like particle size and shape of polymer, temperature, moisture, crystallinity, % D-isomer, residual lactic acid concentration, molecular weight, molecular weight distribution, water diffusion, and metal impurities from the catalyst will affect the polymer degradation rate. Yu and others (2003) have developed a mathematical model to describe the molecular weight and polydispersity index (Q) in PLA thermal degradations. They claimed model ability to predict changes of the molecular weight and polydispersity index in the PLLA thermal degradation. Their model was based on the random chain scission mechanism, effects of temperature, and time on the molecular weight and polydispersity index.

PLA Properties

PLA has unique properties like good appearance, high mechanical strength, and low toxicity; and good barrier properties have broadened its applications. Numerous researchers have studied the different properties of PLA alone and in combination with other polymers as blend or copolymer; and here some of them will be introduced.

Auras and others (2003) studied mechanical, physical, and barrier properties of 2 PLA films by the names of 4030-D, which was made with nominally 98% L-lactide, and 4040-D, which was made with nominally 94% L-lactide resins. Finally, the data from these

2 PLA film samples were compared to those of polystyrene (PS) and polyethylene terephthalate (PET). PLA films showed good tensile strength with higher values than PS but lower than PET. Both 4030-D and 4040-D had lower T_m (melting point) and T_g (glass transition temperature) than PET and PS, which makes PLA better for heat-sealing and thermal processing. In terms of barrier properties of PLA, the permeability coefficients of CO₂ and O₂ were lower than those of PS and comparable to those of PET.

For tensile modulus and flexural modulus, PLA has the highest value in comparison to PS, PP, and HDPE. For notched izod impact (izod impact strength testing is an American society for testing and materials [ASTM] standard method [D256 – 06ae1] of determining impact strength. A notched sample is generally used to determine impact strength. An arm held at a specific height is released. The arm hits the sample and breaks it. From the energy absorbed by the sample, its impact strength is determined), PLA has the lowest one between PS, PP, and HDPE. The elongation at break is low and nearly 4% that is just higher than that of PS (Dorgan and others 2000).

Low glass transition temperature of PLA limits its usages in thermally processed packages. Because of its deformation and its low melting temperature, it is better to use it for heat-sealing and thermoforming applications.

Five major properties of typical biodegradable polymers are compared with LDPE, PS, and PET in Table 3. It is approximately possible to predict the application fields of a polymer by these properties and barrier properties.

The other important property of polymers is their rate of crystallinity. Crystallinity is the indication of amount of crystalline region in the polymer with respect to amorphous content. Crystallinity influences many polymer properties including hardness, modulus, tensile strength, stiffness, crease point, and melting point. So, while selecting a polymer for a required application its crystallinity plays the foremost role.

PLA crystals can grow in 3 structural positions called α , β , and γ forms. They are characterized by different helix conformations and cell symmetries, which develop upon different thermal and/or mechanical treatments. The α form grows upon melt or cold crystallization, the β form develops upon mechanical stretching of the more stable α form, and the γ form, which only recently has been reported to develop on hexamethylbenzene substrate (Di Lorenzo 2005).

Di Lorenzo (2005) measured crystallization rates of PLA over a wide temperature range, using both isothermal and nonisothermal methods. He determined that the crystallization rate of PLA at temperatures between 100 and 118 °C is very high. He concluded that the high crystallization rate of PLA below 120 °C has to be ascribed to the high rate of radial growth of the spherulites (spherical semicrystalline regions inside nonbranched linear polymers).

By modification of the chain architecture through the introduction of branching, different melt flow properties will be obtained. Thermal and rheological properties of 2 commercial types of PLA, linear and branched, were investigated by Dorgan and others (2000). The crystallization kinetic of the branched polymer was faster than that of the linear analog. Longer relaxation times in the terminal region of the branched material introduced it as a higher zero shear rate viscosity. They concluded that by utilizing the structure modifications through polymer branching the ability of using PLA in many processing operations will be extended.

Optical properties of PLA are important in dyeing operations for textiles and in various packaging applications where clarity is desirable. Hutchinson and others (2006) determined the optical

Table 3—Comparison of typical biodegradable polymer properties with LDPE, PS, and PET adapted from Clarinval and Halleux (2005).

	T _g (°C)	T _m (°C)	Tensile strength (MPa)	Tensile modulus (Mpa)	Elongation at break (%)
LDPE	−100	98 to 115	8 to 20	300 to 500	100 to 1000
PCL	−60	59 to 64	4 to 28	390 to 470	700 to 1000
Starch	−	110 to 115	35 to 80	600 to 850	580 to 820
PBAT	−30	110 to 115	34 to 40	−	500 to 800
PTMAT	−30	108 to 110	22	100	700
PS	70 to 115	100	34 to 50	2300 to 3300	1.2 to 2.5
Cellulose	−	−	55 to 120	3000 to 5000	18 to 55
PLA	40 to 70	130 to 180	48 to 53	3500	30 to 240
PHB	0	140 to 180	25 to 40	3500	5 to 8
PHA	−30 to 10	70 to 170	18 to 24	700 to 1800	3 to 25
PHB-PHV	0 to 30	100 to 190	25 to 30	600 to 1000	7 to 15
PVA	58 to 85	180 to 230	28 to 46	380 to 530	−
Cellulose acetate	−	115	10	460	13 to 15
PET	73 to 80	245 to 265	48 to 72	200 to 4100	30 to 300
PGA	35 to 40	225 to 230	890	7000 to 8400	30
PEA	−20	125 to 190	25	180 to 220	400

PGA = Poly(glutamic acid); PEA = Poly(ester amide).

properties of PLA with different amounts of stereoisomer proportions by ellipsometric measurements. They developed an equation for index of refraction of PLA with a wide range of stereoisomer proportions (L-content) within the range of wavelengths from 300 to 1300 nm by using Cauchy coefficients.

There are many PLA resins for different applications with different properties; the general characteristics of a commercial amorphous PLA, injection mold grade and having a 96:4 L:D ratio content, are summarized in Table 4.

PLA barrier properties

One of the most important factors in food packaging polymers is their barrier or permeability performance against transfer of gases, water vapor, and aroma molecules. Gas permeation properties of PLA (L:D ratio 96:4) have been studied by Lehermeier and others (2001) and these values have been reported: at 30 °C, N₂ permeation in PLA was 1.3 (10^{−10} cm³cm/cm²scm Hg), and the activation energy was 11.2 kJ/mol. For oxygen, the corresponding values were 3.3 (10^{−10} cm³cm/cm²scm Hg) and 11.1 kJ/mol. The values for carbon dioxide permeation were 1.2 (10^{−10} cm³cm/cm²scm Hg) and 6.1 kJ/mol. For methane, a value of 1.0 (10^{−10} cm³cm/cm²scm Hg) and an activation energy of 13.0 kJ/mol were found.

The authors concluded that polymer chain branching and small changes in L:D stereochemical content have no effect on permeation properties, but film crystallinity profoundly impacted of the permeation of mentioned gases. For example, due to higher crystallinity of biaxially oriented PLA film, CH₄ permeation is 4.5 times lower than that of the other films. The permeation properties of PLA for all gases studied were very similar to polystyrene.

In research done by Bao and others (2006), different results for pure gas permeation of PLA were obtained, which disagreed with those of previous work. They used a time-lag method for the determination of PLA permeation to pure gases and also determined diffusivity and solubility of N₂, CO₂, and O₂ in PLA film. For example, at 30 °C, N₂ permeability, diffusivity, and solubility in PLA (98.7% L, 1.3% D) were 0.05 (10^{−10} cm³cm/cm²scm Hg), 2.4 × 10^{−8} cm²/s, and 2.2 × 10^{−4} cm³/cm³(polymer) cm Hg, respectively. The measured activation energy of N₂ permeation was 34.6 kJ/mol.

Sorption of nitrogen, oxygen, carbon dioxide, and water in PLA has also been studied at 293.2, 303.2, and 313.2 °K (Oliveira and others 2004).

Shogren (1997) reported that the water vapor transmission rate of crystalline and amorphous PLA in 6, 25, and 49 °C as 27, 82, and 333 g/m² per day for the crystalline form and 54, 172, and 1100 g/m² per day for the amorphous form, respectively. He reported activation energies of 5 and −0.1 kJ/mol for amorphous and crystalline PLA, respectively.

Siparsky and others (1997) used a “solution-diffusion” model to determine the water vapor permeability parameters of different PLA films, PLA copolymers with caprolactone, and blends with polyethylene glycol. These parameters included the solubility coefficient S, which is a measure of the equilibrium water concentration available for hydrolysis and the diffusion coefficient D, which characterizes the rate of water vapor diffusion into the film under specific conditions. They calculated the permeability coefficient by the equation of P = SD. They studied S and D for PLA films by different percent of L and D Lactide. They found the degree of crystalline had little influence on the measured permeability parameters.

In a more detailed research done by Tsuji and others (2006), the effects of D-Lactide content, degree of crystallinity (% X_c), and molecular weight of PLA films on water vapor transfer rate (WVTR) were studied. They observed the WVTR of PLA films decreased monotonically with increasing X_c from 0% to 20%, while leveled off for X_c exceeding 30%; so they suggested this change due to the higher resistance of restricted amorphous regions to water vapor permeation compared with that of the free amorphous regions. They also concluded that changes in M_n of PLA films in the range of 9 × 10⁴ to 5 × 10⁵ g/mol and D-lactide unit content of PLA films in the range of 0% to 50% have insignificant effects on their WVTR values.

Some aforementioned PLA permeability parameters are summarized in Table 5.

Orientation changes the barrier properties. In a study done by Auras and others (2005), oriented PLA (OPLA) was investigated with PET and oriented polystyrene (OPS) with regard to physical, mechanical, and barrier properties. They concluded, in terms of water vapor barrier, that PET gave the best performance, followed by OPS and OPLA. In the case of oxygen barrier properties, PET showed the lowest oxygen permeability coefficients, followed by OPLA and OPS that showed very poor oxygen barrier performance.

According to these results, the barrier properties of PLA are remarkable and better than those of OPS. As a consequence, PLA

Table 4—General characteristics of commercial amorphous poly L-lactid acid film, injection mold grade, 96:4 L:D, produced by NatureWorks® Co.

Characteristics	Unit	Amount	Reference
Physical:			
Molecular weight	g/mol	66000	Garlotta (2001)
Specific gravity	–	1.27	
Solid density	g/cm ³	1.2515	
Melt density	g/cm ³	1.0727	
Glass transition temperature	°C	55	Mehta and others (2005)
Melting temperature	°C	165	
Specific heat (Cp)	J/Kg °C		
190 °C		2060	
100 °C		1955	
55 °C		1590	
Thermal conductivity	W/m °C		
190 °C		0.195	www.natureworksllc.com
109 °C		0.197	(technical data sheet)
48 °C		0.111	
Optical:			
UV light transmission:			Auras and others (2004)
190 to 220 nm		<5%	
225 to 250 nm		85%	
>300 nm		95%	
Visible light transmission		95%	
Color			
L*		90.64 ± 0.21	
a*		–0.99 ± 0.01	
b*		–0.50 ± 0.04	
Mechanical:			
Tensile strength	MPa	59	
Elongation at break	%	7.0	
Elastic modulus	MPa	3500	
Shear modulus	MPa	1287	
Poissons ratio	–	0.3600	www.natureworks.com
Yield strength	MPa	70	(technical data sheet)
Flexural strength	MPa	106	
Unnotched izod impact	J/m	195	
Notch izod impact	J/m	26	
Rockwell hardness	HR	88	
Heat deflection temp.	°C	55	
Vicat penetration	°C	59	
Ultimate tensile strength	MPa	73	
Percent of elongation	%	11.3	
Young's modulus	MPa	1280	
Rehological:			
Cross WLF Viscosity Model:			
n		0.2500	
Tau	Pa	1.00861e + 005	www.natureworksllc.com
D1	Pa-s	3.31719e + 009	(technical data sheet)
D2	K	373.15	
D3	K/P	0	
A1		20.194	
A2	K	51.600	

is suitable for packaging a wide range of foods that are mentioned in the section of PLA applications.

Studies on Migration from PLA

Lactic acid is the lone monomer in the PLA structure and so, migrated agents are lactic acid monomers, dimers, and oligomers. Conn and others (1995) investigated the safety of PLA as a food contact polymer under different conditions and studied the migration of most probable species from PLA. They concluded: (1) Very limited migration can be expected from PLA into foods that it contacts during the intended conditions of use. (2) The small amount of any material that might migrate from PLA into food will be lactic acid, or its dimers (lactoyl lactic acid and lactide)

Table 5—PLA permeability parameters.

		L:D, 96:4 (30 °C) (Lehermeier and others 2001)	L:D, 98.7:1.3 (30 °C) (Bao and others 2006)		
CO ₂	10 ⁻¹⁰ cm ³ cm/ cm ² s cm Hg	1.2	1.1		
O ₂		3.3	0.26		
N ₂		1.3	0.05		
		Water vapor permeation property			
		(Shogren 1997)			
		6 °C 25 °C 49 °C			
Crystalline, 66% crystallinity	g/m ² /day	27	82	333	
Amorphous		54	172	1100	
		(Siparsky and others 1997)			
		20 °C 40 °C 50 °C			
L:D (100:0), 39% crystallinity	cm ³ cm/cm ² s Pa	1.6	1	2	
L:D (100:0), Amorphous		1.9	0.8	2.1	
L:D (95:5)		1.4	2.2	2.1	
L:D (50:50)		2.2	8.7	6.1	

and oligomers that will be subsequently hydrolyzed in aqueous systems to lactic acid. Based on these findings, they concluded that PLA is safe and GRAS for its intended uses in fabricating articles intended for use in contact with food. The authors also mentioned that the projected intake of lactic acid from PLA is approximately 700 times less than the estimated daily lactic acid intake of a breast-fed infant.

Mutsuga and others (2008) determined the PLA migration products for 4 different PLA sheets, which are used in lunch boxes in Japan. They applied 3 food simulants such as water, 4% acetic acid, and 20% ethanol at temperatures of 40, 60, and 95 °C for different periods of time. They concluded that the rate of migration is augmented by high temperatures. The total migrated levels, including lactic acid, lactide, and oligomers, at 40 °C after 180 d were 0.28 to 15 µg.cm⁻² and 0.73 to 2840 µg.cm⁻² for 60 °C after 10 d. The migration test at 95 °C for 30, 60, and 120 min mimicked the use of lunch boxes in a microwave oven at 100 °C or above, and the total migrated levels for 120 min were 2.04 to 49.63 µg.cm⁻².

So, for a PLA much of the concerns about migrations of potential dangerous materials, which exist for petrochemical-based polymers are resolved. These results are only for pure PLA polymer and more studies are needed for its blends and copolymers, also for all the compounds that are applied or added for improving physical, mechanical, and barrier properties of PLA.

PLA Applications

PLA has potential for use in a wide range of applications; Table 6 shows an overview of NatureWorks™ PLA and Ingeo™ fibers, PLA opportunities, and examples of commercially available products. As be seen, PLA food packaging applications are ideal for fresh products and those whose quality is not damaged by PLA oxygen permeability.

PLA is a growing alternative as a “green” food packaging polymer. New applications have been claimed in the field of fresh products, where thermoformed PLA containers are used in retail markets for fruits, vegetables, and salads. The market capacity of these products packaged in PLA is unlimited.

The major PLA application today is in packaging (nearly 70%); the estimation for 2020 shows the increase of other applications especially in fibers and fabrics (Table 7).

Table 6–Business segments for products based on Ingeo™ plastic and Ingeo™ fibers PLA (NatureWorks®) adapted from Vink and others (2004).

Business segment	Commercially available applications
1- Ingeo plastic applications	
Rigid thermoforms	Clear fresh fruit and vegetable clamshells Deli meat trays Opaque dairy (yogurt) containers Bakery, fresh herb, and candy containers Consumer displays and electronics packaging Disposable articles and cold drink cups
Biaxially oriented films	Candy twist and flow wrap Envelope and display carton windows Lamination film Product (gift basket) overwrap Lidding stock Die cut labels Floral wrap Tapes Shrink sleeves Stand-up pouches
Bottles	Cake mix, cereal, and bread bags Short shelf-life milk Edible oils Bottled water
2- Ingeo fiber applications	
Apparel	Casual (sports-), active, and underwear fashion item
Nonwovens	Wipes, hygiene products, diapers, shoe liners, automotive head and door liners, and paper reinforcement
Furnishings	Blankets and panel, upholstery, and decorative fabrics
Industrial carpets	Agricultural and geotextiles ^a Residential/institutional broadloom and carpet tiles
Fiberfill	Pillows, comforters, mattresses, Duvets, and furniture

^aGeotextiles are permeable fabrics which, when used in association with soil, have the ability to separate, filter, reinforce, protect, or drain. Usually geotextiles are placed at the tension surface to strengthen the soil.

Table 7–Main applications of PLA in 2003 and the estimation for 2020 (Wolf 2005).

Sector	Percent of total production (2003)		Estimated percent of total production (2020)	
	Cargill Dow	Hycail	Cargill Dow	Hycail
Packaging	70	70	20	55
Building				
Agriculture	1	12		6
Transportation			20	2
Furniture				
Electric appliance and electronics	1	1	10	10
Houseware		12		6
Other (fibers and fabrics)	28	3 to 5	50	21
Other (analytics)				
Total	100	100	100	100

In the field of packaging, 2 specific areas have received close attention, namely high-value films and rigid-thermoformed containers. PLA brings a new combination of attributes to packaging, including stiffness, clarity, deadfold and twist retention, low-temperature heat sealability, as well as an interesting combination of barrier properties including flavor, and aroma barrier characteristics. The functional properties and benefits of PLA in these areas are presented in Table 8.

Commercialized PLA products demonstrate this fact that PLA is not being used solely because of its degradability, nor because it is made from renewable resources; it is being used because it functions very well and provides excellent properties

Table 8–PLA functional properties for packaging (Kawashima and others 2002).

Functional property	Packaging improvement	Comment
Dead fold, twist, and crimp ^a	Improved folding and sealing	OPLA has excellent dead fold and twist retention
High gloss, and clarity	Package aesthetics	Comparable with PET and cellophane, 3 times more than nylon and PP, 10 times more than LDPE
Barrier properties	Grease and oil resistance	Good resistant to oils and terpens
Renewable resource	Made from CO ₂ and H ₂ O	
Flavor and aroma properties	Reduced taste/odor issues	
Low temperature heat seal	Stronger seals at lower temperatures	PLA can provide an "easy-open" package
High tensile and modulus	Wet paper strength, ability to down guage coating	
Low coefficient of friction, polarity	Printability	Excellent printability, metallizable, antifogging ability
GRAS status	Food contact approved	

^aThe ability to hold a crease or fold, or the ability to retain a twist that is imparted in order to close the edges of the film around a small object.

Table 9–Some commercialized PLA products (Platt 2006; www.natureworkslc.com).

Product	Company name ^a
Packaging	
Films and trays for biscuits, fruit, vegetables, and meat	Treophan, Natura, IPER, Sainsburys, Sulzer, Ecoproducts, RPC
Yogurt cup	Cristallina/Cargill Dow
Rigid transparent packaging of batteries with removable printed film on back side	Panasonic
Trays and bowls for fast food	McDonalds
Envelope with transparent window, paper bag for bread with transparent window	Mitsui, Ecocard
Agriculture and horticulture	
Mulching films	Novamont, Cargill Dow
Long life consumer good	
Apparel (T-shirt, socks)	FILA/Cargill Dow, Kanebo Gosen
Blanket	Ingeo
Casing of walkman	Sony
CD (compact disk)	Sanyo Marvic Media/Lacea
Computer keys	Fujitsu
Small component of laptop housing	Fujitsu/Lacea
Sapre wheel cover	Toyota

^aList is not exhaustive.

at a competitive price. There are many commercialized PLA products in today's market and their variety and consumption are increasing rapidly (Table 9). The reader can find the partners and consumers of PLA from the NatureWorks® Co. website (www.natureworkslc.com).

PLA is also used in biomedical applications, with various uses as internal body components mainly in the of restricted load for example, interference screws in ankle, knee, and hand; tacks and pins for ligament attachment; rods and pins in bone, plates and screws for craniomaxillofacial bone fixation (Lim and others 2003); and also for surgical sutures, implants, and drug delivery systems (Furukawa and others 2005; Mills and others 2006).

PLA as an Active Packaging Material

Release studies from PLA

Active packaging is defined as an intelligent or smart system that involves interactions between package or package components and food or internal gas atmosphere and complies with consumer demands for high quality, fresh-like, and safe products (Labuza and Breene 1989).

Active packaging is an innovative approach to change the condition of the packaging to extend shelf-life or improve safety or sensory properties while maintaining the quality of the food. Traditional packaging concepts are limited in their ability to prolong shelf-life of food products. The most important active packaging concepts are O₂ and ethylene scavenging, CO₂ scavengers and emitters, moisture regulators, antimicrobial packaging concepts, antioxidant release, release or adsorption of taste, and aroma molecules (Vermeiren and others 1999; Lopez-Rubio and others 2004; Kerry and others 2006).

As a GRAS and biodegradable material, and also because of its biosorbability and biocompatible properties in the human body, PLA and its copolymers (especially poly-glycolic acid) is attractive to pharmaceutical and medical scientists as a carrier for releasing various drugs and agents like bupivacaine (Sokolsky-Papkov and others 2009), rapamycin (Miao and others 2008), melittin (Cun and others 2008), 5-fluorouracil (Liu and others 2008), amoxicillin (Xu and Czernuszka 2008), human nerve growth factor (rhNGF) (Gu and others 2007), and gentamicin (Schnieders and others 2006) and many others.

In food domains, little research has been done studying the ability of PLA as an active packaging material. PLA is a relatively new polymer and needs time to become an acceptable and an effective active packaging in the market.

Antioxidants have been added to food packaging material for the intentional purpose of migration into food, because prooxidant effects are often seen to a high extent and could be reduced by antioxidants. Van Aardt and others (2007) studied the release of antioxidants from loaded poly (lactide-co-glycolide) (PLGA) (50:50) films, with 2% α -tocopherol, and a combination of 1% butylated hydroxytoluene (BHT) and 1% butylated hydroxyanisole (BHA), into water, oil (food simulant: Miglyol 812), and milk products at 4 and 25 °C in the presence and absence of light. They concluded that in water medium PLGA (50:50) showed hydrolytic degradation of the polymer and release of BHT into water. In Miglyol 812, no degradation or antioxidant release took place, even after 8 wk at 25 °C. Milk fat was stabilized to some extent when light-exposed dry whole milk and dry buttermilk were exposed to antioxidant-loaded PLGA (50:50). The authors also suggested potential use of degradable polymers as a unique active packaging option for sustained delivery of antioxidants, which could be a benefit to the dairy industry by limiting the oxidation of high-fat dairy products, such as ice cream mixes.

PLA and antimicrobial packaging trends

The innovative strength of PLA antimicrobial packaging has a direct impact on consumer health by creating safer and more wholesome packaged foods. Active packaging realizes certain extraordinary and vital functions other than providing an inert barrier between product and external conditions.

Active substances that are important and considered for novel bioactive packaging include antimicrobials, vitamins, phytochemicals, prebiotics, marine oils, and immobilized enzymes (Lopez-Rubio and others 2006).

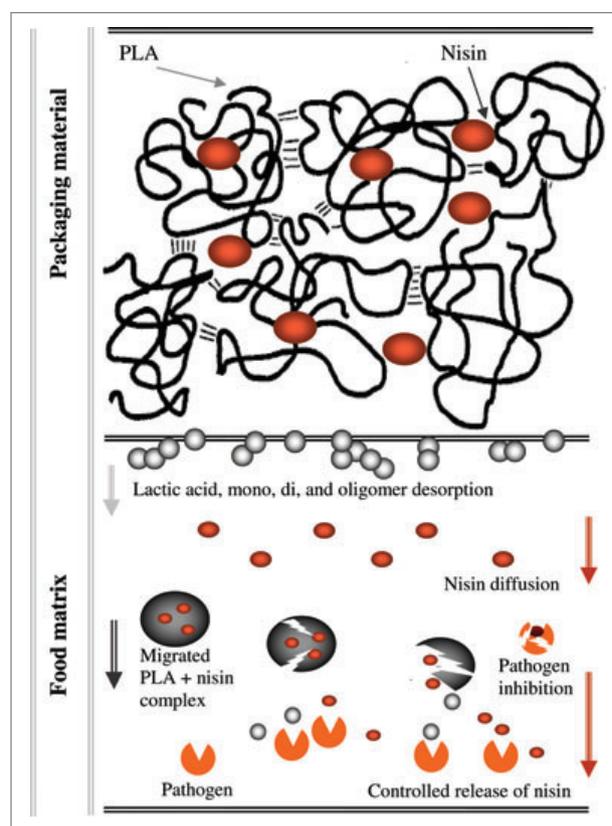


Figure 3—Schematic representation of PLA film with nisin as an active agent incorporated and release thereof.

A whole range of active additives, including silver-substituted zeolite, organic acids and their salts, bacteriocins such as nisin and pediocin, enzymes such as lysozyme, a chelator like ethylenediaminetetraacetic acid (EDTA), lactoferrin, and plant extracts have already been successfully incorporated in antimicrobial active packaging (Joerger 2007).

The most widely used bacteriocin in active food packaging is nisin due to its GRAS status (FDA 2001). Successful introduction of a new active packaging requires careful attention to the interactions in the active agent, packaging, and food triangle.

Notion of controlled release. In particular for active packaging, the major complexity emerges for migration/diffusion as either slow release of package component itself or as an active agent being incorporated. In both contexts, the evaluation of materials compliance with regulations includes migration monitoring for package component (monomer) and additives (active agent). A schematic representation is shown in Figure 3 for release of an active agent (nisin) alone and in conjunction with packaging material. Recently, the predictive mathematical modeling for active agent-controlled release and its various approaches were excellently reviewed by Poças and others (2008).

On the contrary, to study an additive's release from a package, active agent desorption from the multilayer biodegradable film and diffusion in agarose gels were monitored. The data attained after 2 or 6 d of contact between antimicrobial films and agarose gels were employed to find out nisin mass transfer by numerical modeling following Fick's 2nd law. The values were in the range from 0.87×10^{-3} m/s to 4.30×10^{-3} m/s and 6.5×10^{-11} m²/s to 3.3×10^{-10} m²/s, for nisin apparent desorption and diffusion

coefficients, respectively. The diffusion process was governed by interactions between nisin, package, and food matrix simulant (Chollet and others 2009).

Mode of incorporation. The customized direct incorporation of active agents may result in a loss of activity due to interactions with food components, thus showing from a diminution of active concentration and dilution into bulk foods (Kim and others 2002; Coma 2008). The incorporation of antimicrobial agents into PLA packaging material slows down their release and helps to maintain high concentrations of the active compounds against pathogenic bacteria like *Listeria monocytogenes* (Jin and others 2009).

In the last decade, the above-mentioned slow release approach has been used for PLA. In this regard, major antimicrobial agents include bacteriocins, predominantly nisin (Ariyapitipun and others 1999; Jin and others 2009), lactic acid (Ariyapitipun and others 1999), lysozyme (Del Nobile and others 2009), and chitosan (Torres-Giner and others 2008).

Novel PLA active packaging potential approaches

Although the above-mentioned PLA systems reduced resistant bacterial strain development and guaranteed a higher level of microbial protection for certain food products, their casting and preparation was complicated and inactivation of the active proteins was observed. Active agent modification by attachment to a polymer did not yield biologically active derivatives. Up till now, a literature study reveals that relatively low attention has been given to micro-encapsulated active agents in foods. Active agent-loaded polymeric micro-/nanoparticles give the impression of being promising formulations to achieve long-lasting antimicrobial activity (Salmaso and others 2004; Sanchez-Garcia and others 2007).

Thus, this particular controlled release concept can be enlarged to the applications of other active agents like antioxidants for oil-rich foods and antisticking/antifogging agents for cheese slices and fresh fruits, respectively.

PLA Modifications

The special characteristics of PLA can make it a good fit for some applications but may also require modifications for some others. For example, the oxygen and moisture permeability of PLA is much higher than for most other plastics, such as PE, PP, and even PET. However, the applications of PLA are limited by several factors such as low glass transition temperature, weak thermal stability, and low toughness and ductility (Harada and others 2007).

For extending PLA applications, the properties like impact strength or flexibility, stiffness, barrier properties, thermal stability, and production costs must be improved. Generally, modifiers have been studied to improve stiffness at elevated temperatures, reduce cost, or increase the degradation rate of PLA.

Some efforts of PLA modifications in the field of packaging are presented in Table 10.

A large number of investigations have been performed on the blending of PLA with various polymers, for example, thermoplastic starch, poly (ethylene oxide), poly (ethylene glycol), poly (ϵ -caprolactone), poly (vinyl acetate), poly (hydroxy butyrate), cellulose acetate, poly (butylene succinate), and poly (hexamethylene succinate). Low molecular weight compounds have also been used as plasticizers for PLA, for example, oligomeric lactic acid, glycerol, triacetate, and low molecular weight citrates (Ljungberg and others 2005).

The choice of polymers or plasticizers to be used as modifiers for PLA is limited by the requirements of the application. For

packaging and hygiene applications, only nontoxic substances approved for food contact and personal care can be considered as plasticizing agents. The plasticizer should be miscible with PLA, thus creating a homogeneous blend. The plasticizer should not be too volatile, because this would cause evaporation to occur at the high temperature used during processing. Furthermore, the plasticizer should not be prone to migration into the materials in contact with the plasticized PLA. It would also cause the blended materials to regain the brittleness of pure PLA (Ren and others 2006).

There is a tendency for plasticizers to migrate to the surface of a polymer. A possible way to prevent this migration would be to increase the molecular weight of the plasticizers.

However, increasing the molecular weight too much would eventually decrease the solubility causing phase separation and formation of a 2-phase system.

The final properties of these blends depend on the chemical structure of the original components, the mixing ratio of the constituent polymers, the interaction between the components, and the processing steps to which they are then subjected.

Amorphous PLA exhibits lower modulus above the glass transition temperature and poor heat resistance, which limits the wide application of PLA in the general plastic use. Thus, how to improve the crystallization behavior or enhance the degree of crystallinity (% Xc) of PLA becomes the main problem that must be solved. Decreasing the cooling rate of PLA from melt and providing an annealing process for PLA articles is believed to be the most efficient way to enhance the (% Xc) of PLA. It has been reported that the smaller the cooling rate, the higher the (% Xc). Annealing endows PLA chain segments enough activation energy and promotes the crystallization through the reorganization process. Especially, in a certain condition, the annealing process also induces the polymorphic transition in PLA (Li and others 2009).

Other kinds of modifications, such as surface modifications, are being applied in biomedical uses for improving polymer release properties (Janorkar and Hirt 2004; Koo and Jang 2008).

Nanotechnology and PLA Food Packaging

Nanotechnology and its applications in food science have recently been studied by several researchers. The use of nanoparticles, such as micelles, liposomes, nanoemulsions, biopolymeric nanoparticles, and cubosomes, as well as the development of nanosensors aimed at ensuring food safety, are some novel nano-food applications.

Nanoparticles can be used as bioactive compounds in functional foods. Bioactive compounds that can be found naturally in certain foods have physiological benefits and might help to reduce the risk of certain diseases, including cancer. Omega-3 and omega-6 fatty acids, probiotics, prebiotics, vitamins, and minerals have found their applications in food nanotechnology as bioactive compounds (Sozer and Kokini 2009).

Nanotechnology is also applicable in food packaging in the form of elementary components of food packaging. This approach includes improving packaging performances like its gas, moisture, ultraviolet, and volatile barriers, increasing mechanical strength, decreasing weight, and increasing the heat resistance and flame retardancy of the packaging material. Nanoadditives, intelligent packaging (using nanosensors), delivery and controlled release of nutraceuticals, antibacterial agents, self-cleaning packaging, and systems to monitor product conditions during transportation are other novel nano-approaches in food packaging (Ray and Bousmina 2005; Sozer and Kokini 2009).

Table 10–Summary of PLA modifications for packaging applications.

Type of modification	Treatment or added material	Effect	Reference
Modifier	Citrate esters	Lowering the Tg and improving the elongation at break	Labrecque and others (1997)
	Triacetate or tributyl citrate	Decrease in Tg and increase in crystallinity	Ljungberg and Wesslén (2002)
	Oligomeric malonate esteramides	Decrease in Tg and improvement of the strain at break	Ljungberg and others (2005)
	4,4-Methylene diphenyl diisocyanate	Tg value increased to 64 °C, tensile strength increased from 4.9 to 5.8 MPa good nucleating agent for PLA crystallization	Li and Yang (2006)
	Polyglycerol esters	Improving the elongation at break	Uyama and others (2006)
	Polyethylene glycol and acetyl triethyl citrate	Decrease in Tg and increase in crystallization rate	Li and Huneault (2007)
	Talc Bifunctional cyclic ester Poly(1,3-butylene adipate)	Increase the ductility at more than 10% Enhance PLA toughness Decrease in storage modulus and glass transition temperature but increase in elongation at break	Li and Huneault (2007) Jing and Hillmyer (2008) Wang and others (2008)
Blending with:	Polycarbodiimide	Improve the thermal stability at 210 °C for up to 30 min	Yang and others (2008)
	Polyvinyl acetate	Increase in tensile strength and percent elongation	Gajria and others (1996)
	Poly ethylene oxide (PEO) Poly ϵ -caprolactone (PCL) Poly ethylene glycol (PEG)	Elongation at break of more than 500% High improvement in mechanical properties Enhance the crystallinity of PLA and biodegradability	Nijenhuis and others (1996) Tsuji and Ikada (1996) Sheth and others (1997)
	Starch with different plasticizers	Lowering the price, decreasing Tg, and increasing crystallinity and biodegradability	Ke and Sun (2001); Jacobsen and Fritz (1996); Ke and others (2003)
	Polyvinyl alcohol and starch Ethylene vinyl alcohol (EVOH)	Increase in tensile strength Improvement of mechanical, thermal, and biodegradability properties	Ke and Sun (2003a) Lee and others (2005)
	Polycarbonate	Improvement of mechanical properties and biodegradation rate	Wang and others (2007)
	Poly ethylene glycidyl methacrylate (PEGMA)	Production of super-tough PLA materials	Oyama (2009)
Copolymerization of PLA and:	DL-mandelic acid	Increasing Tg and improving mechanical properties	Kylmä and others (1997)
	ϵ -Caprolactone	Improving the decomposition temperatures and crystallinity	Park and others (1998)
	Polyvinyl chloride Acrylonitrile–butadiene–styrene	Improving strength and toughness Improved impact strength and elongation at break with a slight loss in modulus and tensile strength	Lu and others (2008) Li and Shimizu (2009)
Physical treatment	Vacuum compression-molding and solid-state extrusion techniques	Flexural strength and flexural modulus were improved up to 221 MPa and 8.4 GPa, respectively	Lim and others (2001)
	Orientation	Significant improvement in tensile and impact properties	Grijpma and others (2002)
	Annealing	Increasing the toughness	Park and others (2004)
	Aging Drawing	Increasing the Tg Improvement in tensile and fracture properties	Quan and others (2004) Todo (2007)

Addition of different fillers to polymers for improving their performances like their strength and stiffness, barrier properties, resistance to fire and ignition, and also decreasing their price has always been a common objective in packaging technology. Traditionally, mineral fillers such as clay, silica, and talc are incorporated in film preparations in the range of 10% to 50% by weight to reduce film cost or to improve its performance in some way. However, mechanical strength of such films, in general, decreases when fillers are present. Recently, nanocomposites have received significant attention as an alternative to conventional filled polymers (Rhim 2007).

Nanocomposites

Nanocomposites are a new class of composites that are particle-filled polymers for which at least 1 dimension of the dispersed particles is in the nanometer range. Three types of nanocomposites include isodimensional nanoparticles (with 3 nano dimensions), nanotubes or whiskers (with 2 nano dimensions), and polymer-

layered crystal nanocomposites (with 1 nano dimension) (Alexandre and Dubois 2000).

Although several nanoparticles have been recognized as possible additives to enhance polymer performance, the packaging industry has focused its attention mainly on layered inorganic solids like clays and silicates, due to their availability, low cost, significant enhancements, and relative simple processability (Azeredo 2009). These nanocomposites exhibit markedly improved mechanical, thermal, optical, and physicochemical properties when compared with the pure polymer or conventional (microscale) composites. The layered silicates commonly used in nanocomposites consist of 2-dimensional layers, which are 1-nm thick and several microns long depending on the particular silicate (Alexandre and Dubois 2000).

The commonly used layered silicates for the preparation of polymer-layered silicate (PLS) nanocomposites are montmorillonite (MMT), hectorite, and saponite (Sinha Ray and Okamoto 2003).

The matrix of polymer/clay nanocomposites consists mainly of synthetic polymers including thermosets such as epoxy, thermoplastics like poly (methyl methacrylate), nonpolar polymers like polyethylene and polypropylene, polar polymers like nylon, and conductive polymers like polyaniline. In addition, biodegradable polymers such as PLA and polycaprolactone (PCL) have also been tested for the manufacture of nanocomposites with layered silicate (Rhim 2007).

PLA nanocomposites

The combination of PLA and montmorillonite-layered silicate may result in a nanocomposite with good barrier properties that is suitable for film packaging material. The modulus of PLA would be increased by the addition of montmorillonite. However, the incorporation of the montmorillonite clay into PLA could decrease the toughness of the PLA composites. There are various technical approaches to achieve a balance of good strength and toughness for PLA nanocomposites. The addition of poly ethylene glycol could act as a good plasticizer in a PLA/clay systems (Shibata and others 2006).

A comprehensive review is provided by Sinha Ray and Okamoto (2003) for the preparation, characterization, materials properties, crystallization behavior, melt rheology, and foam processing of pure polylactide (PLA) and PLA/layered silicate nanocomposites. They concluded this new family of composite materials frequently exhibits remarkable improvements in its material properties when compared with those of virgin PLA. Improved properties can include a high storage modulus both in the solid and melt states, increased tensile and flexural properties, decreased gas permeability, increased heat distortion temperature, and increased rate of biodegradability of pure PLA.

In a complementary review, Sinha Ray and Bousmina (2005) presented recent developments on the above-mentioned properties for many biodegradable polymers' nanocomposites. They described 2 types of biodegradable polymers: (a) originating from renewable sources like PLA, poly (3-hydroxybutyrate), thermoplastic starch (TPS), plant-based polymers, cellulose, gelatin, or chitosan; and (b) originating from petroleum sources like poly (butylene succinate), aliphatic polyesters, poly(ϵ -caprolactone), or poly (vinyl alcohol).

Recent research on PLA nanocomposites

The potential applications of PLA-based nanocomposites are in food packaging, medical applications, and tissue cultures. Some research conducted on PLA nanocomposites in the field of food packaging after the year 2005 is presented here.

Biodegradability of polymers through photodegradation has been studied by using TiO₂ nanoparticles as photocatalysts that decompose various organic chemicals like aldehyde, toluene, and polymers such as PE, PP, PVC, and PS.

In a study done by Nakayama and Hayashi (2007), TiO₂ nanoparticles were prepared and the surface of TiO₂ was modified using propionic acid and *n*-hexylamine, with the modified TiO₂ uniformly dispersed into PLA matrixes without aggregation. They studied the PLA-TiO₂ nanocomposite's photodegradation under UV light and concluded photodegradability of nanocomposites can be efficiently promoted.

Melt intercalation is a method where the blending of polymer and silicate layers is followed by molding to form a polymer-layered silicate nanocomposite. In general, for intercalation, polymers and layered hosts are annealed above the softening point of the polymer. Chow and Lok (2009) used this method for study-

ing the effect of maleic anhydride-grafted ethylene propylene rubber (EPMgMA) on the thermal properties of PLA/organo-montmorillonite nanocomposites. They concluded that the addition of OMMT (Organo-montmorillonite) and EPMgMA did not influence much the T_g and T_m (melting temperature) of PLA nanocomposites. The degree of crystallinity of PLA increased slightly in the presence of OMMT; it had been supposed that OMMT could act as a nucleating agent to increase the crystallinity of PLA. In contrast, the addition of EPMgMA may restrict the crystallization process and crystal formation of PLA, which subsequently reduces the degree of crystallinity of PLA/OMMT nanocomposites. Finally, they claimed that the thermal stability of PLA/OMMT was greatly enhanced by the addition of EPMgMA.

Kim and others (2009) studied the effect of bacterial cellulose on the transparency of PLA/bacterial nanocomposites, since bacterial cellulose had shown good potential as reinforcement or preparing optically transparent materials due to its structure, which consists of ribbon-shaped fibrils with diameters in the range from 10 to 50 nm. They found that light transmission of the PLA/bacterial cellulose nanocomposite was quite high due to the size effect of the nanofibrillar bacterial cellulose. Additionally, the tensile strength and Young's modulus of the PLA/bacterial cellulose nanocomposite were increased by 203% and 146%, respectively, compared with those of the PLA.

Carbon nanotubes (CNTs) have been the subject of much attention because of their outstanding performance including excellent mechanical, electrical, and thermal properties. The most promising area of nanocomposite research involves the reinforcement of polymers using CNTs as reinforcing filler (Kim and others 2007).

Li and others (2009) introduced functionalized multiwalled carbon nanotubes (f-MWCNTs) into PLA to investigate the effect of such filler on the crystallization behavior of PLA. They concluded that the addition of f-MWCNTs accelerates the crystallization of PLA dramatically and induces formation of homogeneous and very small spherulites. The results of polarized optical microscopy showed that the average spherulite diameter is about 200 μ m, but for nanocomposites it was very difficult to differentiate the spherulites one by one.

Numerous studies have also been done on PLA nanocomposites in medical science regarding drug delivery systems, tissue engineering, and bone fixation (Jo and others 2004; Sakata and others 2006; Chen and others 2007).

PLA Degradability, Biodegradability, and Recyclability

Almost all the conventional plastics such as PE, PP, PS, and PVC are resistant to microbial attack; on the contrary aliphatic polyesters like PLA are readily degraded by microorganisms present in the environment. According to ASTM D6400-04, a biodegradable plastic is "a plastic that degrades because of the action of naturally occurring microorganisms such as bacteria, fungi, and algae," and a compostable plastic is "a plastic that undergoes degradation by biological processes during composting to yield carbon dioxide, water, inorganic compounds, and biomass at a rate consistent with other known compostable materials and leaves no visually distinguishable or toxic residues.

PLA degradation was studied in animal and human bodies for medical applications like implants, surgical sutures, and drug delivery materials (Vainionpaa and others 1989). In these environments, PLA is initially degraded by hydrolysis and the soluble oligomers formed are metabolized by cells. PLA degradation upon disposal in the environment is more challenging because PLA is largely resistant to attack by microorganisms in soil or sewage under

ambient conditions. The polymer must 1st be hydrolyzed at elevated temperatures (about 58 °C) to reduce the molecular weight before biodegradation can commence. No degradation was observed on PLA sheets after 6 wk in soil, thus PLA will not degrade in typical garden compost (Brandrup and others 1999; Ohkita and Lee 2006). Urayama and others (2002) reported that the molecular weight of PLA films with different optical purity of the lactate units (100% L and 70% L) decreased by 20% and 75%, respectively, after 20 mo in soil.

Kale and others (2007) studied the degradation of PLA bottles in a real composting condition (compost containing cow manure, wood shavings, and waste feed) at 65 °C for 30 d. They observed major fragmentation, which produces decomposition of the polymer chain into shorter oligomer chains and monomers since the 4th day, and on the 15th day, the bottles were already in pieces and mostly consisted of parts from cap threads, and neck (bottle parts having higher thickness) and finally on the 30th day the bottles were completely degraded.

Microbial and enzymatic degradation of PLA have recently been studied by many researchers because these types of degradations usually do not need the high temperatures to be accomplished. Williams (1981) 1st reported the degradation for PLLA by proteinase K from *Tritirachium album.*, afterward many studies were done for finding different enzymes corresponding PLA degradation. Reported enzymes that enable to degrade PLA in different scale include, alkaline protease (Oda and others 2000), serine proteases such as subtilisin, trypsin, elastase, and α -chymotrypsin (Lim and others 2005), Cutinase-like enzyme (Masaki and others 2005). Lipase could hydrolyze low molecular weight PLLA and some copolymers such as PDLA (poly D,L-lactic acid) and, poly(D-lactid-co-glycolide) but not PDLA (poly D-lactic acid) and high molecular weight PLLA (Fukuzaki and others 1989). Pranamuda and others (2001) found an enzyme from *Amycolatopsis* sp. cultures and named it PLLA depolymerase. The optimum pH and temperature for this enzyme were 6.0 and 37 to 45 °C, respectively. PLLA depolymerase can also hydrolyze casein, silk fibroin, succinyl-*p*-nitroanilide, but not PHB and PCL. The enzymatic degradation of aliphatic polyesters by hydrolysis is a 2-step process. The 1st step is adsorption of the enzyme on the surface of the substrate through surface-binding and the 2nd step is hydrolysis of the ester bond (Tokiwa and Calabia 2006).

Pranamuda and others (2001) 1st isolated a PLA-degrading microorganism of *Amycolatopsis* strain from soil environment, which was capable of degrading 60% of the PLA film after 14 d. Suyama and others (1998) reported that PLA-degrading microorganisms are not widely distributed in the natural environment and, thus, PLA is less susceptible to microbial attack in the natural environment than other synthetic aliphatic polyesters like PHB, PCL, and Poly(butylenes succinate) (PBS). Several PLA-degrading microorganisms, their enzymes, and substrate specificities are reported in Table 11. Upon disposal in the environment, PLA is hydrolyzed into low molecular weight oligomers and then mineralized into CO₂ and H₂O by the microorganisms present in the environment.

Microbial degradation of PLA should be studied for packaging of foods containing microorganisms including lactic acid bacteria, and fungi for their probable abilities of PLA degradation. Torres and others (1996) reported the ability of assimilation of lactic acid and racemic oligomer products of PLA for 2 strains of *Fusarium moniliforme* (widely distributed in soil) and on strain of *Penicillium roqueforti* (the main fungus in blue cheese, and can be isolated from soil).

Recycling diverts material from alternative waste streams such as land filling or incineration, as well as conserves natural resources and energy. PET and HDPE make up a large percentage of the plastic bottles that get recycled. Sorting PLA in recycling facilities is difficult due to low volumes and in many cases, the PLA container looks like PET. Because of this, the possibility of mixing the different materials together exists. As a result, there is concern in the recycling community that PLA bottles, at high enough levels, would contaminate the PET recycle stream due to chemical and thermal property differences. The National Association for PET Container Resources (NAPCOR) recently announced its concern for potential contamination of the PET recycling stream associated with PLA bottles. This trade association for the PET plastic industry in the U.S. and Canada cited its concerns involving cost of separation, increased contamination, yield loss, and impact on recycled PET (RPET) quality and processing (www.napcor.com).

Consequently, NatureWorks® and Primo Water Corp. conducted a commercial scale bottle recycling evaluation to demonstrate that automated systems being used today in the recycling industry are capable of separating PLA bottles from PET bottles with good accuracy and efficiency (93%). In this evaluation, near-infrared equipment was used since it is a common sorting technology in large recycling operations and can accurately identify many different types of polymers (NatureWorks® 2009).

Recycled bottles crushed, chopped into flakes, and pressed into bales. They enter to final recycling step and are changed to PLA monomers; L-lactic acid or L-lactide. There are 2 methods for PLA recycling, primarily hydrolysis or solvolysis to L-lactic acid or L-lactic acid-based compounds and, 2nd, depolymerization to the cyclic dimer, L-lactide. Both methods have problems with low yield of monomers in a short period and require the removal of catalysts and additives used for hydrolysis, solvolysis, or depolymerization (Tsuji and others 2003).

High-temperature hydrolysis, normally above the melting point, is an effective way to hydrolyze PLA rapidly to L-lactic acid without the aid of catalysts. The highest maximum yield of L-lactic acid (about 90%) in a high temperature and high pressure water was attained at 250 °C for 10 to 20 min (Tsuji and others 2001).

Conclusion

In previous years, the most negative point of PLA was its price in comparison with petrochemical-based polymers. Today, by using other sources of dextrose, optimizing lactic acid production processes and its costs, substituting electricity energy by wind and solar energy for PLA production, optimizing PLA production processes, and increasing PLA demands, reduction of its price can be attained. The present PLA price is much lower than in previous years, but it is not fixed and it even will be considerably lower in the future because, according to expert forecasts, beyond 2010 the global demand for biodegradable plastics will continue to increase by 30% each year and PLA will take a large part of this market because of its valuable properties (Bastioli 2005).

The linkage of a 100% bio-originated material and nanomaterials opens new windows for becoming independent from petrochemical-based polymers and also free of environmental and health concerns.

Substituting PET with PLA in food packages, which require high-barrier properties, is not feasible unless some modifications are applied to develop its permeability. Also, the brittleness of PLA may also limit its applications where toughness and impact resistance are critical. However, with the help of nanotechnology

Table 11—PLA-degrading microorganism, their enzymes substrate specificities, and detection methods used in degradation tests.

Microorganism	Enzyme	Substrate specificity	Detection method for PLA degradation
<i>Amycolatopsis</i> sp. strain HT 32	Protease	L-PLA	Film-weight loss; monomer production (lactic acid)
<i>Amycolatopsis</i> sp. strain 31 18	Protease	L-PLA	Film-weight loss; monomer production
<i>Amycolatopsis</i> sp. strain KT-s-9	Protease	Silk fibroin, L-PLA	Clear-zone method
<i>Amycolatopsis</i> sp. strain 41	Protease	L-PLA, silk powder, casein, Suc-(Ala)3-pNA	Film-weight loss; monomer production
<i>Amycolatopsis</i> sp. strain K104-1	Protease	L-PLA, casein, fibrin	Turbidity method
<i>Lentzea waywayandensis</i> (formerly <i>Saccharothrix waywayandensis</i>)	Protease	L-PLA	Film-weight loss; monomer production
<i>Kibdelosporangium aridum</i>	Protease	L-PLA	Film-weight loss; monomer production
<i>Tritirachium album</i> ATCC 22563	Protease	L-PLA, silk fibroin, elastin	Film-weight loss; monomer production
<i>Brevibacillus</i> (formerly <i>Bacillus brevis</i>)*	Protease	L-PLA	Change in molecular weight and viscosity
<i>Bacillus stearothermophilus</i> *	Protease	D-PLA	Change in molecular weight and viscosity
<i>Geobacillus thermocatenulatus</i> *	Protease	L-PLA	Change in molecular weight and viscosity
<i>Bacillus sinithii</i> strain PL 21*	Lipase (Esterase)	L-PLA, pNP-fatty acid esters	Change in molecular weight
<i>Paenibacillus amylolyticus</i> strain TB-13	Lipase	DL-PLA, PBS, PBSA, PES, PCL, triolein, tributyrin	Turbidity method
<i>Cryptococcus</i> sp. strain S-2	Lipase (Curtinase)	L-PLA, PBS, PCL, PHB	Turbidity method

Adapted from Tokiwa and Calabia (2006).

and providing safe PLA nanocomposites, many of its weakness compared to petrochemical-based polymer will be resolved.

According to its safety, biodegradability, and ability for being improved in a tailor-made fashion, the authors predict the substituting of many petrochemical-based polymers by PLA for almost all pharmaceutical and direct food contact packaging materials in the near future.

Nomenclature

- ASTM = American society for testing and materials;
 EVOH = Ethylene vinyl alcohol;
 GPPS = General purpose poly(styrene);
 HDPE = High-density poly(ethylene);
 HIPS = High-impact poly(styrene);
 LDPE = Low-density poly(ethylene);
 LLDPE = Linear low-density poly(ethylene);
 MMT = Montmorillonite;
 MWCNT = Multiwalled-carbon nanotube;
 OMMT = Organo-montmorillonite;
 OPLA = Oriented poly(lactic acid);
 OPP = Oriented poly(propylene);
 OPS = Oriented poly(Styrene);
 PBAT = Poly(butylene adipate terephthalate);
 PBS = Poly(butylenes succinate);
 PBST = Poly(butylene succinate terephthalate);
 PC = Poly(carbonate);
 PCL = Poly(ϵ -caprolactone);
 PEA = Poly(ester amide);
 PEG = Poly(ethylene glycol);
 PEGMA = Poly(ethylene-glycidyl methacrylate);
 PEO = Poly(ethylene oxide);
 PET = Poly(ethylene terephthalate);
 PGA = Poly(glutamic acid);
 PHB = Poly(3-hydroxybutyrate);
 PHV = Poly(hydroxyl valerate);
 PLLA = Poly(L-lactic acid);
 PP = Poly(propylene);
 PS = Poly(styrene);
 PTMAT = Poly(tetramethylene adipate terephthalate);
 PVA = Poly(vinyl alcohol);
 PVC = Poly(vinyl chloride);
 TPS = Thermoplastic starch.

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III . Résultats et Discussion

Chapitre 3.1

Effets des Antioxydants Phénoliques Synthétiques sur les Propriétés Structurales, Mécaniques et Barrières du Film d'Acide Poly Lactique

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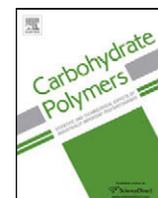
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Résumé

Des antioxydants phénoliques synthétiques (APS), tels que le BHA, BHT, PG et TBHQ ont été ajoutés (1%, w/w) à un film d'acide poly lactique (PLA). Les APS n'ont aucun effet significatif sur la transition vitreuse et la température de fusion du PLA, mais ont réduit la cristallinité de film d'environ 26% et la température de cristallisation de 6-12°C à partir de la valeur de 107°C trouvée pour PLA pur. Le module d'Young du film de PLA a été réduit d'environ 12% par le BHA et d'environ 17% pour les PG et TBHQ. La résistance à la traction du film a également été influencée par les BHA, PG et TBHQ. La perméabilité à la vapeur d'eau du film de PLA n'a pas été modifiée par l'addition des APS et la perméabilité à l'oxygène n'a augmenté que de 30% ajout de BHT. Aucun changement significatif n'a été observé pour l'énergie de surface et l'hydrophilicité de surface du film de PLA. La microstructure du film de PLA étudiée par la microscopie à force atomique a changé de manière significative en termes de rugosité (Ra et Rq). Les résultats ont montré que l'addition d'APS dans le PLA pour la création d'emballages actifs n'a eu aucun effet négatif sur propriétés techniques des films.



Effects of synthetic phenolic antioxidants on physical, structural, mechanical and barrier properties of poly lactic acid film

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ABSTRACT

Synthetic phenolic antioxidants (SPAs) including BHA, BHT, PG and TBHQ were added (1%, w/w) to a poly (lactic acid) (PLA) film. SPA addition had no significant effect on the glass transition and melting temperatures of the PLA film but reduced the crystallinity about 26% and the crystallization temperature by 6–12 °C from the value of 107 °C found for pure PLA film. Young's modulus of PLA film was reduced about 12% by BHA and about 17% by PG and TBHQ. The tensile strength of PLA film also was influenced by BHA, PG and TBHQ. Water vapor permeability of PLA film was not changed by addition of SPAs and PLA oxygen permeability only increased by 30% on BHT addition. No significant changes were observed for surface energy and surface hydrophilicity of PLA films. PLA film microstructures investigated by Atomic force microscopy were significantly changed in term of roughness parameters (R_a and R_q). The results showed that the addition of SPAs in PLA for the creation of active packaging had no negative effect on PLA technical properties.

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1. Introduction

Poly (lactic acid) (PLA) is a highly versatile, biodegradable, aliphatic polyester made from 100% renewable resources, such as corn and sugar beets following fermentation of starch and condensation of lactic acid. PLA offers great promise in a wide range of commodity applications and competes with polystyrene for many food packaging applications. It is a highly transparent and rigid material with a relatively low crystallization rate that makes it a promising candidate for the fabrication of biaxially oriented films, thermoformed containers and stretch-blown bottles (Dorgan, Lehermeier, & Mang, 2000; Yew, Mohd Yusof, Mohd Ishak, & Ishiaku, 2005). Comparing to some petro-chemical based polymers, PLA has some weaknesses; the oxygen and moisture permeability of PLA is much higher than for most other plastics, such as PE, PP, and even PET. PLA applications are also limited by several factors such as low glass transition temperature, weak thermal stability, and low toughness and ductility (Harada et al., 2007). PLA has the ability to be tailor-made for the properties needed for special applications (Arvanitoyannis, 1999).

There are many research studies focusing on PLA modification for extending PLA applications. Properties like impact strength or flexibility, stiffness, barrier properties and thermal stability can be improved by the addition of modifiers, blending, copolymerizing and physical treatments. Citrate esters, triacetate, tributyl citrate, oligomeric malonate esteramides, 4,4-methylene diphenyl diisocyanate, polyglycerol esters, polyethylene glycol, acetyl triethyl citrate, talc, bifunctional cyclic ester, poly(1,3-butylene adipate) have been studied as PLA modifiers (Jamshidian, Tehrani, Imran, Jacquot, & Desobry, 2010).

The additives used for PLA modification are limited by the requirements of the application. For food packaging and hygiene applications, only nontoxic substances approved for food contact and personal care can be considered as modifying agents. Additionally, the modifiers should be miscible with PLA, thus creating a homogeneous blend but should not be too volatile because this would cause evaporation to occur at the elevated temperatures used at processing. The migration of modifiers from PLA should also be considered to minimize the contamination of the food in contact with the modified PLA (Ljungberg & Wesslén, 2002). As migration of additives from polymers in contact with foodstuffs is not usually zero, so one logical way of modifying PLA is to use compounds which are usually consumed in foods such as permitted natural or synthetic antioxidants, natural antimicrobial agents like nisin, and so on. In this case if the modifying agents migrate

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Table 1
Summary of researches based on impregnation of antioxidants to polymers.

Object	Antioxidant(s)	Polymer	Reference
Migration to food simulants	BHT	HDPE	Till et al. (1982)
Mobility in polymer in contact with fatty food simulants	α -Tocopherol, BHT	LDPE	Wessling, Nielsen, Leufvén, and Jagerstad (1998)
Retention in polymer in contact with foodstuffs and food-simulating liquids	α -Tocopherol	LDPE, PP	Wessling, Nielsen, Leufvén, and Jagerstad (1999)
Influence of antioxidant on the polymer properties	α -Tocopherol	LDPE	Wessling, Nielsen, and Leufvén (2000)
Stability of antioxidant in packaging film used for oatmeal	α -Tocopherol, BHT	LDPE	Wessling et al. (2001)
Migration of phenolic antioxidants from branch and linear polymer	Santonox, Irganox 1081, Low inox 22M46	PE	Lundback, Hedenqvist, Mattozzi, and Gedde (2006)
Antioxidant migration study of some polymers	BHA, BHT, AO 2246, Ethanox 330, Irganox 1010, Irganox 1076	LDPE, HDPE, PP, PVC, PET	Dopico-García et al. (2007)
Antioxidant migration into food simulants	Irgafos 168, Irganox 1076	LLDPE	Jeon, Park, Kwak, Lee, and Park (2007)
Release of antioxidants to dry milk products and food simulants liquids	α -Tocopherol, BHA, BHT	PLGA	Van Aardt et al. (2007)
Release of antioxidant to asadero cheese and its effect on oxidation and odor stability	BHT	LDPE	Soto-Cantú et al. (2008)
Improving antioxidant release by smart blending	α , β , and δ -tocopherols	LLDPE:HDPE	Schaich, Obinata, and Yam (2008)
Release of antioxidant from polymer film to whole milk powder	α -Tocopherol, BHA, BHT	LLDPE:PP LLDPE:PS HDPE-EVA-LDPE (co-extruded)	Granda-Restrepo, Peralta, Troncoso-Rojas, and Soto-Valdez (2009)
Release of antioxidant from polymer film to distilled water	L-Ascorbic acid, L-tyrosine	CA	Gemili, Yemenicioglu, and Altinkaya (2010)

HDPE, high density polyethylene; LDPE, low density polyethylene; LLDPE, low linear density polyethylene; PE, poly ethylene; PP, poly propylene; PVC, poly vinyl chloride; PET, polyethylene terephthalate; PLGA, poly(lactide-co-glycolide); EVA, ethylene vinyl acetate; CA, cellulose acetate.

to food, the safety concerns regarding to release of unpermitted agents do not occur and released compounds can act as preservatives resulting in some types of active packaging. The addition of synthetic antioxidants in polymer formulations is a common practice as a way to protect polymeric degradation during processing and also during their life-time. Antioxidants protect the polymer against the formation of undesirable oxidative and low molecular compounds which can migrate to foods causing a decrease in food quality. However, the potential migration of these substances to the packaged product has been an issue that should be considered to maintain food quality and safety (Dopico-García, Loípez-Vilarinõ, & González-Rodríguez, 2007; Garde, Catala, Gavara, & Hernandez, 2001).

Oxidation produces low-molecular-weight off-flavor compounds, destroys essential nutrients, and produces toxic compounds and dimmers or polymers of lipids and proteins (Aruoma, 1998). In fats and oils, antioxidants delay the onset of oxidation or slow the rate of oxidizing reactions. The main purpose of using an antioxidant as a food additive is to maintain the quality of that food and to extend its shelf life rather than improving the quality of the food. Since free radicals were found to be responsible for lipid oxidation, hundreds of natural and synthetic compounds have been evaluated for their efficacy as radical scavengers or for their other inhibitory effects. Among them, only four synthetic antioxidants are widely used in foods; namely, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) (Wanasundara & Shahidi, 2005).

Apart from the modifying effects of antioxidants for polymers, antioxidant-active packaging is an important category of active packaging and promising technique for extending food shelf life, different antioxidants have been impregnated in several polymer films and their migrations in real foods and food simulants have been studied. In some cases, the effects of migrated antioxidants

on lipid oxidation and odor stability have also been investigated (Table 1), but in most studies important objectives were rates, effects, and mechanisms of antioxidants' release. Mechanical, physicochemical and barrier properties affect technical properties and stability of polymer in various conditions and structural properties can influence the rate and amount of active agents whom desired to migrate. The first group of properties is routinely examined in using modifying compounds but structural properties are rarely studied. Atomic force microscopy (AFM) technique can provide precise information about morphological changes made by modifiers which can affect their release to food stuffs (Kikkawa, Fujita, Abe, & Doi, 2004; Sanchez-Garcia, Gimenez, & Lagaron, 2008). X-ray photoelectron spectroscopy (XPS) determines chemical composition of the surface layer and characterizes possible chemical bonding between the modifying agent and the polymer (Kiss, Bertóti, & Vargha-Butler, 2002).

Recently, Byun, Kim, and Whiteside (2010) studied the addition effect of α -tocopherol and polyethylene glycol 400 (PEG 400) on film-cast-extruded PLA. The authors concluded that the addition of PEG 400 as a plasticizer decreases the T_g , and tensile strength of the film and reversely increases % elongation at break. They showed the incorporation of α -tocopherol into PLA film increases oxygen permeability and decreases water vapor permeability (WVP) of the PLA film. Lopez-Rubio and Lagaron (2011) characterized UV stability and mechanical properties of some biopolyesters including PLA, PCL (polycaprolacton) and polyhydroxybutyrate-co-valerate (PHBV) containing β -carotene. They showed a plasticizing effect of β -carotene resulting in significant increase in the deformation at break and lower Young's modulus. The results confirmed even though UV radiation degraded β -carotene in polyesters but their mechanical properties remained more stable than those of the pure polymers suggesting β -carotene as a natural additive to increase the UV stability of biopolyesters. The authors used Raman

spectroscopy to study the dispersion and degradation of β -carotene in selected areas of the different biopolyester films.

The addition of active compounds may affect the structure and other physical and mechanical characteristics which are important in respect to technological and functional aspects of packaging materials, so an active packaging without desired applied properties will be useless. However, the authors believe that the first step in creating a successful active packaging is to identify the effects and interactions between active compound and polymer structure which may be inapplicable in some cases. To our knowledge the effects of synthetic phenolic antioxidants (SPAs) on PLA physical, mechanical and structural properties in one hand for its modification and in the other hand for providing an antioxidant-PLA packaging have not been well investigated before, so the aim of this study focused on characterizing the addition effects of SPAs including BHA, BHT, PG, and TBHQ on physical, structural, mechanical, thermal, and gas barrier properties of PLA.

2. Materials and methods

2.1. Materials

Poly (lactic acid) (PLA), 2002 D, was purchased in pellet form from Natureworks® Co., Minnetonka (USA); Chloroform D (99.8% CDCL₃) from Euriso-Top (France); Magnesium nitrate, phosphorus pentoxide, BHA, BHT, PG, and TBHQ from Sigma–Aldrich (France); Chloroform HPLC grade and potassium nitrate from VWR international (France).

2.2. Film preparation method

For each film containing 1% of antioxidant, a solution of 3.5% (w/w) of PLA was prepared in chloroform and afterwards 1% (w/w) of antioxidants based on PLA dry matter were added to solution and mixed for 1 h for complete solution of antioxidants in polymer matrix. Then 20 g of polymer solution was cast in Teflon 90 × 110 mm Petri dishes (Welch, USA) the extra chloroform was evaporated in a dark place under hood during 24 h at 25 °C then films were placed in incubator (Mettler, Germany) at 30 °C for 7 days for complete solvent evaporation. The Petri dishes were kept in a hermetic container containing P₂O₅ powder before each analysis. The final film thickness was 100 ± 5 μm measured (at least 8 points) by a mechanical micrometer (Messmer, UK) according to ASTM D374.

2.3. Determination of SPAs in PLA films

The amounts of SPAs in PLA films after drying were measured by nuclear magnetic resonance spectroscopy (NMR). ¹H NMR chemical shifts in ppm were collected at 600.13 MHz on an Avance III 600 spectrometer (Bruker, Germany) at 298 K. PLA films were dissolved in CDCL₃ and SPAs amount were quantified by comparing the peak area of each antioxidant in a sample with the one of the corresponding peak of the standard at a known concentration. Standard precautions regarding quantization in NMR were taken including identical spectrometers settings, repetition time between two consecutive scans long enough in comparison with relaxation times. All tests were accomplished in triplicate.

2.4. Thermal properties

Thermal analysis for measurement of transition temperatures including glass transition temperature (T_g) and melting temperature (T_m), and enthalpies of fusion (ΔH_f) and crystallization (ΔH_c) was carried out with a differential scan calorimeter (NETZSCH, Germany). Specimens weighing 10 mg were sealed in an aluminum

pan and heated under protective nitrogen (40 ml min⁻¹) at the rate of 10 °C min⁻¹ from 5 to 210 °C then cooled to 5 °C and reheated to 210 °C with the same cooling–heating rate.

T_g , T_c and T_m were determined from the obtained thermogram. The degree of crystallinity was evaluated according to Eq. (1):

$$\% \text{Crystallinity} = 100 \times \frac{\Delta H_m - \Delta H_c}{\Delta H_f} \quad (1)$$

where ΔH_f is the enthalpy of fusion; ΔH_c is the enthalpy of crystallization; and ΔH_m is the enthalpy of fusion of a wholly crystalline PLA (93.6 J/g) (Arnoult, Dargent, & Mano, 2007).

2.5. Mechanical properties

A Lloyd instruments testing machine (AMETEK, UK) was used to determine different mechanical properties including tensile strength, Young's modulus and percent of elongation at break according to ASTM D882. Before testing, all samples were equilibrated for 48 h at 50 ± 2% relative humidity (RH) in a container using magnesium nitrate saturated solution at 20 ± 1 °C. Equilibrated film specimens were mounted in the film-extending grips of the testing machine and stretched at a rate of 25 mm min⁻¹ until breaking. The RH and temperature of the testing environment was held at 52 ± 2% and 20 ± 2 °C, respectively.

2.6. Water vapor permeability (WVP) measurement

The Water vapor transition rate (WVTR) measurements were done using a Permatran W3/31 (Mocon, Inc., Minneapolis, MN, USA) according to ASTM standard F1249 (ASTM, 1995). The calibration of the instrument for WVTR measurements was performed using polyester standard films provided by Mocon. Water vapor concentration within the nitrogen stream is measured by an infrared detector contained within the instrument. Samples were exposed to 90% RH and tested at 38 ± 1 °C. Water vapor permeability (kg m⁻² s⁻¹ Pa⁻¹) was calculated by multiplying the WVTR by film thickness. The tests were done in triplicate and the mean values were reported.

2.7. Oxygen permeability measurement

The oxygen transition rate (OTR) measurements were accomplished using an OX-TRAN 2/21 (Mocon, Inc., Minneapolis, MN, USA) according to ASTM standard D3985 (ASTM, 1995). Measurements were conducted at 23 ± 1 °C and 0% RH. Flat films were clamped into the diffusion cell that was purged of residual oxygen using an oxygen-free air carrier gas. The carrier gas was routed to the sensor until a stable zero was established. Pure (99.9%) oxygen was then introduced into the outside chamber of the diffusion cell. Oxygen permeability (m³ m m⁻² s⁻¹ Pa⁻¹) was calculated by multiplying the OTR by film thickness. Tests were done in triplicate with the mean values were reported.

2.8. Surface characterization

2.8.1. Measurement of contact angle

The contact angle of the films was obtained at 20 °C using a commercial Digidrop meter (GBX Scientific Instruments, France) equipped with a charge-coupled device (CCD) camera 2/3" and a Nikon 60 mm macro lens. This apparatus comprises the software named Windrop⁺⁺ which precisely determines the drop size of the liquid placed on the sample surface. The contact angle was measured by adding randomly 10 drops of ultra pure liquids (2 μl) on the surface of a film disk (80 mm in diameter). Using Windrop⁺⁺ software provided with instrument, measurements of the static contact angle values were fully automated.

2.8.2. Measurement of surface energy

The total surface energy of PLA films were graphically determined using same Digidrop meter apparatus by the method of Owens–Wendt which usually used for solids with low surface energy such as polymers. For this measurement, 4 special liquids including, water, glycerol, ethylene glycol and diiodomethane were used. The Owens–Wendt theory divides the surface energy into two components: surface energy due to dispersive interactions and surface energy due to polar interactions. Owens and Wendt proved that the total surface energy of a solid, γ_s , can be expressed as the sum of contributions from dispersion γ_s^d and polar γ_s^p force components ($\gamma_s = \gamma_s^d + \gamma_s^p$). These can be determined from the contact angle, θ , data of polar and non-polar liquids with known dispersion γ_L^d and polar γ_L^p parts of their surface energy, via Eq. (2) (Awaja, Gilbert, Kelly, Fox, & Pigram, 2009; Michalski, Desobry, & Hardy, 1998):

$$\gamma_L(1 + \cos \theta) = 2\sqrt{\gamma_L^d \gamma_s^d} + 2\sqrt{\gamma_L^p \gamma_s^p} \quad (2)$$

where γ_L is the liquid surface tension.

2.8.3. X-ray photoelectron spectroscopy (XPS)

The XPS analyses were carried out with a Kratos Axis Ultra spectrometer (Kratos Analytical, Manchester, UK) using a monochromatic Al K α source. All spectra were recorded at 90° take-off angle and analyzed area was about 700 $\mu\text{m} \times 300 \mu\text{m}$. Survey spectra were recorded with 1.0 eV step and 160 eV analyzer pass energy and the high resolution regions with 0.1 eV step and 20 eV pass energy, with an exception for the carbon with 0.05 eV step. In both cases the hybrid lens mode was employed (magnetic and electrostatic). During the data acquisition the Kratos charge neutralizer system was used on all specimens with the following settings: filament current 2 A, charge balances 3.5 V, filament bias 1.0 V and magnetic lens trim coil 0.34 A. The C–(C, H) carbon was set to 284.60 eV and therefore used as an internal energy reference.

Spectra were analyzed using the Vision software from Kratos (Vision 2.2.6). A Shirley base line was used for the subtraction of the background from each peak. Quantification was performed using the photoemission cross sections and transmission coefficients given in the Vision package.

2.8.4. Atomic force microscopy analysis (AFM)

The surface morphology was analyzed by using a D3100 AFM equipped with a Nanoscope 5 electronic from Veeco manufacturer. The images were recorded at ambient conditions (20 °C and 30% RH) and in soft intermittent contact mode (IC-AFM or Tapping™ AFM). Tap150 tapping mode cantilevers (Veeco model No MPP-12100) with a typical spring constant of about 5 N/m and a resonance frequency around 165 kHz was used for scanning. Tapping force was controlled by the ratio between set point amplitude (A_{sp}) and free-air amplitude (A_0). The scan rate was adjusted in the range of 0.5–1 Hz depending on the image quality. Each scan line contains 512 pixels and a whole image is composed of 512 scan lines. For acquisition of the surface morphology amplitude error, phase and height images were recorded on several areas of film surface. Only the representative height images were presented here for discussion with a scan size of 20 μm^2 , 10 μm^2 , 5 μm^2 and 2 μm^2 for PLA–BHT, PLA–BHA, PLA and PLA–PG, and PLA–TBHQ, respectively.

All offline image flattening and analyses of the images were conducted at the software environment provided by the AFM manufacturer. The statistical parameters related with sample roughness (ASME B46.1, 1995) were estimated by the software equipment in a 2 μm^2 area at the center of AFM image, so average roughness (R_a) and root mean square roughness (R_q) were only presented.

Table 2

Thermal properties of PLA films containing 1% SPAs.

	T_g	T_c	T_m	% Cryst.
PLA–BHA	54.3 \pm 1.0 ^a	98.4 \pm 3.6 ^{ac}	143.4 \pm 0.7 ^a	13.8 \pm 1.5 ^a
PLA–BHT	55.1 \pm 0.9 ^a	97.6 \pm 0.8 ^{ac}	143.3 \pm 0.1 ^a	14.3 \pm 0.6 ^a
PLA–PG	55.8 \pm 0.8 ^a	100.4 \pm 2.8 ^c	140.1 \pm 0.1 ^b	14.8 \pm 1.62 ^a
PLA–TBHQ	54.6 \pm 0.8 ^a	95.3 \pm 1.8 ^a	140.8 \pm 1.1 ^b	14.1 \pm 1.1 ^a
Pure PLA	55.3 \pm 0.7 ^a	107.0 \pm 0.8 ^b	144.7 \pm 0.3 ^c	19.5 \pm 0.5 ^b

The means of three replicates plus or minus the standard deviation are shown. Significant difference ($P \leq 0.05$) is represented by different alphabetic letters.

2.9. Statistical analysis

The data were analyzed by analysis of variance (ANOVA) using StatGraphics (version 16, StatPoint Technologies, Inc., Warrenton, VA, USA) and differences among mean values were processed by the Student–Newman–Keuls multiple range test. Multiple comparisons were performed through 95% least significant difference intervals ($P \leq 0.05$).

3. Results and discussion

3.1. SPAs amounts in PLA films

NMR data showed decrease amounts of SPAs in PLA films. PLA films loaded with 1% (wt%) SPAs yielded a recovery of 90%, 87%, 85% and 80% for TBHQ, BHT, BHA, and PG respectively. The 23% lost of BHA and BHT in poly(lactide-co-glycolide) (PLGA) films due to their evaporation has been reported (Van Aardt et al., 2007). The volatility of antioxidants is desired for dried food applications because of their rapid migration via evaporation from polymer surface to target food (Wessling, Nielsen, & Giacini, 2001).

3.2. Thermal properties

Fig. 1 presents a DSC thermograph of pure PLA, PLA–BHA and PLA–TBHQ films. DSC results of five different PLA films containing 1% SPAs and pure PLA are presented in Table 2. The pure PLA exhibited a T_g around 55 °C and a melting temperature (T_m) around 144 °C. Calculated PLA crystallinity according to equation 1 was 19.5% and crystallization temperature (T_c) around 107 °C. In each DSC curve, only a single exothermic crystallization peak, T_c , appears which suggests a single crystallization process during the dynamic cooling procedure. Therefore, the distribution of the crystallites size such a melt-crystallized sample is believed to be unimodal instead of bimodal. Consequently, the occurrence of double melting peaks during the subsequent heating scan is not associated with the possibility of bimodal size distribution of the crystallites (Ling & Spruiell, 2006). The double melting peak of PLA has been attributed to a melting-recrystallization phenomenon where the lower endotherm corresponds to the melting of initially present crystals. The partially molten material is thought to be able to recrystallize into more perfect α -crystals polymorph melting at higher temperature in the second endotherm (Sarasua, Zuza, Imaz, & Meaurio, 2008). The double melting endotherm was more pronounced for films containing SPAs demonstrating the various effect of SPAs on melting-recrystallization behavior, for example the height of first melting peak of PLA–BHA is smaller than the second one while for PLA–TBHQ the height of first melting peak is larger than the second one.

The addition of the antioxidants reduced the crystallization temperature by 6–12 °C from the value of 107 °C found for pure PLA film. All SPAs equally reduced the crystallinity by about 26%; they also slightly reduced the T_m but had no significant effect on T_g . It seems that SPAs somehow hinder polymer chains lateral rearrangements and hence crystallization in PLA. These similar effects

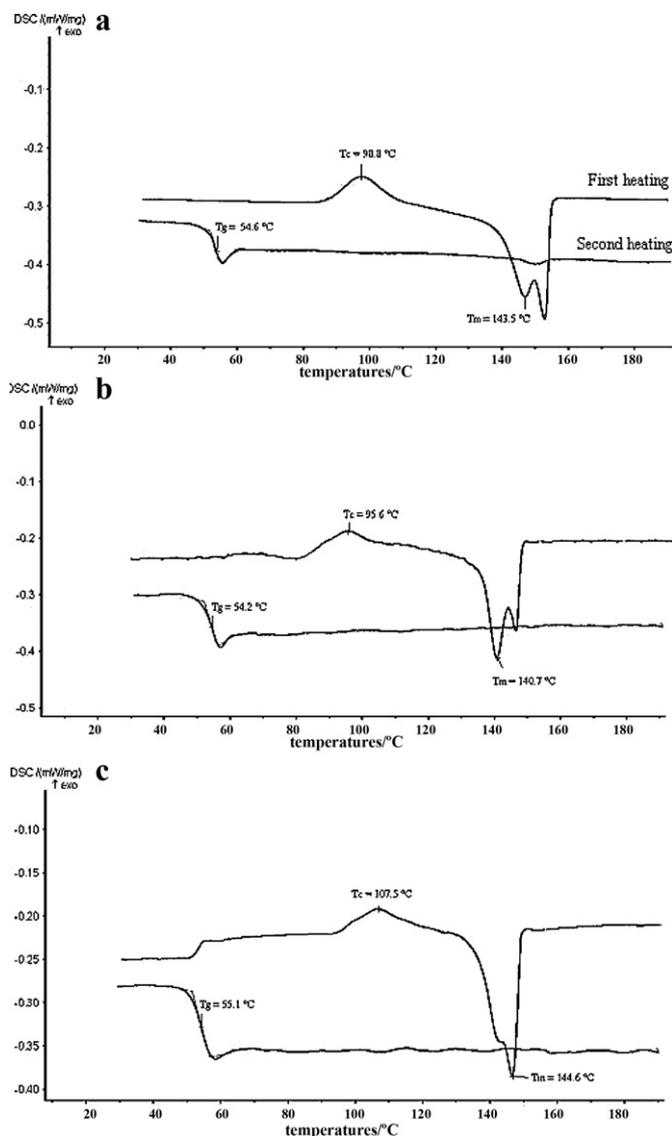


Fig. 1. DSC thermograph of PLA films, a = PLA-BHA, b = PLA-TBQ and c = pure PLA.

were observed for cellulose fiber and β -carotene in PCL and PHBV, respectively (Lopez-Rubio & Lagaron, 2011; Sanchez-Garcia et al., 2008). Reduction of crystallinity is a negative point in the aspect of mechanical and barrier properties and positive point for PLA rapid biodegradation in nature or in biomedical applications. In many applications, increasing the crystallization amount of PLA is desired because in its amorphous form, the range of application of PLA is severely limited by its low glass transition temperature. Thus for these cases the crystalline form is required to increase the high temperature stability of PLA (Li & Huneault, 2007). Addition of modifiers such as 4,4-methylene diphenyl diisocyanate (Li & Yang, 2006), increases T_g of PLA and other studies confirmed some modifications in PLA thermal properties by blending or copolymerizing (Dorati et al., 2007; Jang, Shin, Lee, & Narayan, 2007; Quan, Liao, & Zhao, 2004).

3.3. Mechanical properties

Young's modulus, tensile strength and % elongation at break are three important factors for studying the mechanical properties of polymeric films. The mechanical properties of PLA films containing 1% SPAs is represented in Table 3. Young's modulus and tensile

Table 3
Mechanical properties of PLA films containing 1% SPAs.

	Young's modulus (GPa)	Tensile strength (MPa)	Elongation at break (%)
PLA-BHA	1.96 \pm 0.10 ^a	49.6 \pm 3.5 ^{ac}	3.4 \pm 0.2 ^a
PLA-BHT	2.17 \pm 0.07 ^b	54.7 \pm 3.5 ^{bc}	3.0 \pm 0.4 ^a
PLA-PG	1.85 \pm 0.08 ^a	46.9 \pm 2.6 ^a	3.2 \pm 0.4 ^a
PLA-TBHQ	1.85 \pm 0.08 ^a	45.8 \pm 3.2 ^a	3.3 \pm 0.2 ^a
PLA	2.24 \pm 0.04 ^b	58.0 \pm 2.8 ^b	3.6 \pm 0.2 ^a

The means of at least six replicates plus or minus the standard deviation are shown. Significant difference ($P \leq 0.05$) is represented by different alphabetic letters.

strength of PLA film were reduced by addition of BHA, PG and TBHQ. Young's modulus was reduced 12% by BHA and 17% by PG and TBHQ. These three antioxidants had also a reducing effect on tensile strength; 14% by BHA and 20% by PG and TBHQ. Only BHT had no negative effect on PLA mechanical properties. It is well known that PLA crystallinity can play a significant role in the mechanical and durability performance in rigid molded applications. An increase in the polymer's overall crystallinity can lead to improvements in stiffness, strength, heat deflection temperature and chemical resistance (Harris & Lee, 2008). PLA crystallinity has also a direct effect on tensile strength and Young's modulus properties; as be seen in Table 2 the PLA crystallinity is reduced by SPAs and the relation between crystallinity and tensile strength of PLA films containing 1% SPAs is well demonstrated in Fig. 2, in reducing the % crystallinity Young's modulus and tensile strength were decreased. Changes in polymer matrix or reduction of polymer homogeneity due to SPAs addition can also affect these mechanical parameters and could be the reason of these behaviors. In the case of PLA-BHT Young's modulus and tensile strength were not changed despite of reduced amount of crystallinity proposing the participation of other factor(s) made by BHT addition on PLA structure neutralizing the negative effect of crystallinity reduction. Complementary studies using microscopy methods can provide more information about BHT incorporation in PLA matrix. SPAs did not change the % elongation at break presenting no plasticizing effect of SPAs on PLA. Rhim, Mohanty, Singh, and Ng (2006) showed the PLA films prepared by the thermocompression method had higher modulus and strength but lower toughness than PLA films prepared by solvent-casting method. They showed that the elongation at break of PLA films prepared by solvent-casting and thermocompression is about 203% and 3%, respectively. This high value of elongation was reported due to existence of chloroform (about 13%) in solvent-cast film which acts as a plasticizer. In this study the film solution drying prolonged for 7 days at 30 °C ensured no solvent existence in the final films and no plasticizing effect of chloroform.

The addition effects of different compounds on PLA mechanical properties have been largely studied; citrate esters (Labrecque, Kumar, Davé, Gross, & McCarthy, 1997), oligomeric malonate

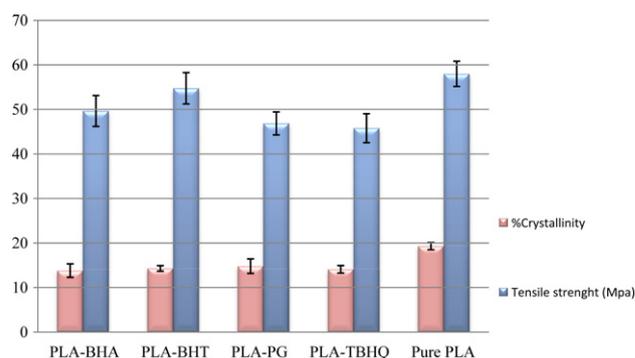


Fig. 2. Tensile strength and % crystallinity of PLA and PLA films containing 1% SPAs.

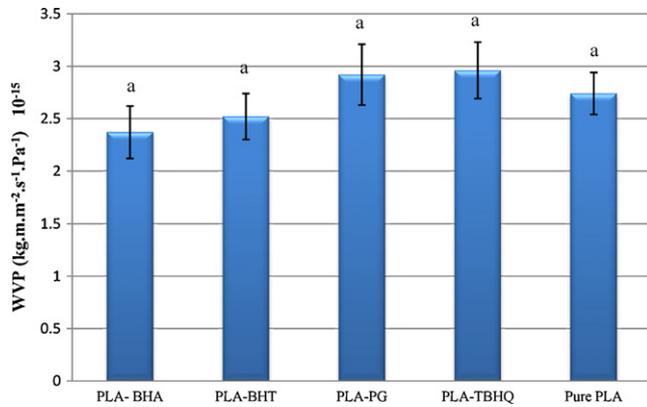


Fig. 3. WVP of PLA and PLA films containing 1% of SPAs at 38 °C and RH=90% (all measurements were performed in triplicate).

esteramides (Ljungberg, Colombini, & Wessleén, 2005), 4,4-methylene diphenyl diisocyanate (Li & Yang, 2006), bifunctional cyclic ester (Jing & Hillmyer, 2008), poly(1,3-butylene adipate) (Wang, Zhang, Yu, & Fang, 2008) have been shown to have the ability to modify the tensile strength values of PLA. Blending, copolymerizing and physical treatment have also been studied to increase the tensile strength of PLA (Gajria, Davé, Gross, & McCarthy, 1996; Grijpma, Altpeter, Bevis, & Feijen, 2002; Kylmä, Härkönen, & Seppälä, 1997; Li & Shimizu, 2009; Nijenhuis, Colstee, Grijpma, & Pennings, 1996; Todo, 2007). However, there are always some differences in mechanical and barrier properties of solvent-cast and extruded films.

3.4. WVP

WVP is one of the most important properties in food packaging mainly because of the significant role of water in deteriorative reactions and also microbial growth. Water acts as a solvent or carrier, causes textural degradation, and provides chemical and enzymatic reactions. WVP of PLA and PLA films containing 1% SPAs are shown in Fig. 3. SPAs addition had no significant effect on WVP of PLA. Crystallinity has an important effect on WVP because the crystallites themselves are impermeable. Thus, water vapor must seek out amorphous zones in order to penetrate in polymer. In the case of SPAs the 26% reduction of crystallinity had no noticeable influence on WVP. Tsuji, Okino, Daimon, and Fujie (2006) showed that the water vapor transition rate (WVTR) of PLA films decreased monotonically with increasing the crystallinity (X_c) from 0 to 20%, while leveled off for X_c exceeding 30%. They assumed that this is probably due to the higher resistance of “restricted” amorphous regions to water vapor permeation compared with that of the “free” amorphous regions.

3.5. Oxygen permeability

The oxygen permeability of PLA films containing 1% SPAs is presented in Fig. 4. Oxygen permeability of extruded PLA given in literature data is between 1.9×10^{-18} and $6.0 \times 10^{-18} \text{ m}^3 \text{ m m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$ for various experimental setups (Auras, Harte, & Selke, 2004; Auras, Singh, & Singh, 2005; Colomines, Domenek, Ducruet, & Guinault, 2008; Sanchez-Garcia, Gimenez, & Lagaron, 2007). Our results even though obtained from solvent cast films are consistent with those data. BHT increased PLA oxygen permeability by 30%. The bars in oxygen permeability graph imitate the tensile strength and Young's modulus bars showing the influence of BHT on PLA structure, while BHA, PG and TBHQ did not change the PLA oxygen permeability. Like WVP, oxygen permeability decreases with increasing crystallinity in polymers.

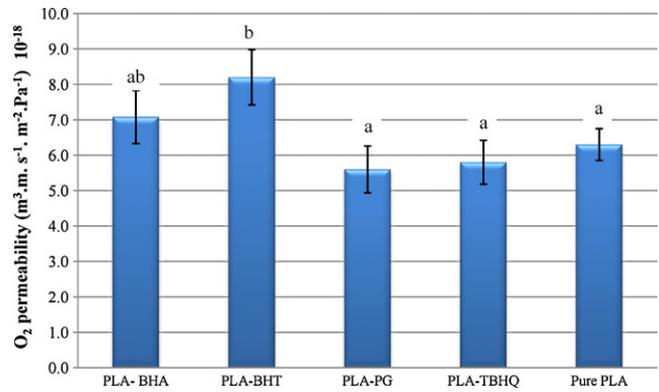


Fig. 4. Oxygen permeability of PLA films containing 1% of SPAs at 23 °C and RH=0% (all measurements were performed in triplicate, significant difference ($P \leq 0.05$) is represented by different alphabetic letters).

Generally, this is because gases cannot diffuse and permeate in the crystalline phase and small molecules only pass through the amorphous region because the polymer chain mobility is considerably restricted in the crystalline phase, leading to difficult diffusion and dissolution (George & Thomas, 2001). The decrease about 26% of PLA crystallinity caused by antioxidant could not have a great effect on oxygen permeability because all PLA films containing antioxidants have a similar crystallinity but PLA-BHT has a higher oxygen permeability explaining other influencing factors. In some cases the crystallinity has a reverse effect leading to higher oxygen permeability because of the phenomenon of de-densification of the amorphous phase which counteracts the decrease of the quantity of permeable amorphous phase due to crystallization (Liu, Hu, Schiraldi, Hiltner, & Baer, 2004). Colomines et al. (2008) reported that the quenched PLA film containing 39% crystallinity had higher oxygen permeability than PLA film containing 2% crystallinity; so they hypothesized the PLA de-densification during quenching treatment.

By considering the SPAs molecular structure and their physicochemical properties such as molar volume and surface area it is possible to interpret the BHT effect on oxygen permeability. One factor which can play an important role is antioxidant volume and therefore its occupied space in polymeric network; more occupied space provides less polymer orientation and more oxygen permeability. Molecular volumes of SPAs were calculated by Molecular Modeling Pro™ 5 (Fig. 5). As BHT has a more open structure so its molecular volume is higher than other antioxidants facilitating the oxygen passage. Using Henry's law describing the gas solubility in a polymer and Fick's laws that characterize the gas diffusion in that material, the gas permeability may be expressed as a function of both solubility and diffusion coefficients, so it can be proposed that BHT increases oxygen diffusion in PLA film. A little increment in oxygen permeability influenced by BHA can be also attributed to its higher molecular volume rather than PG and TBHQ.

3.6. Contact angle and surface energy

The surface energy of a polymer influences its adhesive properties, and is important for processes such as printing and heat sealing. The contact angle, surface energy, polar component and dispersive component of PLA films with and without antioxidants have been presented in Table 4. As can be observed in this table, the contact angle of the PLA film with antioxidant slightly increases from 75.3° to the highest value 78.4° for PLA-TBHQ. This increment can be made because of hydrophobic nature of these antioxidants. The influence of SPAs on contact angle is not great because of two reasons, firstly because of their little added amounts (1%) to PLA

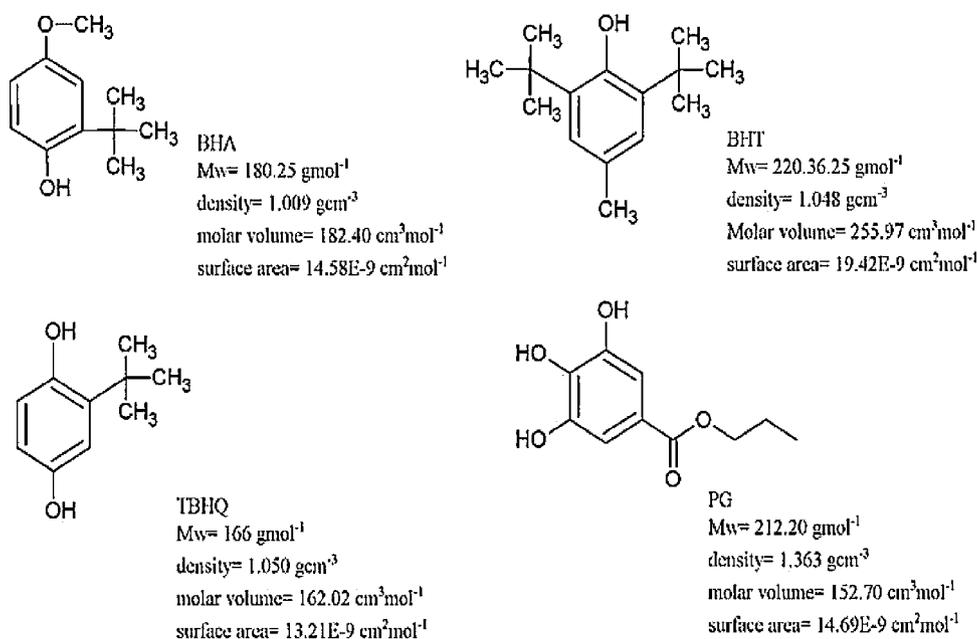


Fig. 5. Chemical structure and properties of synthetic phenolic antioxidants (molar volume and surface are calculated by Molecular Modeling Pro™ 5).

and secondly because their total distribution occurred throughout the polymer network not only on its surface. The light increase in PLA contact angle by adding the SPAs, demonstrates the decreased wettability, suggesting that the PLA surfaces contain an increased number of hydrophobic groups after adding these antioxidants. The PLA hydrophobicity is desired for printing practices and plasma treatments is a usual way for modifying this property (Chaiwong, Rachtanapun, Wongchaiya, Auras, & Boonyawan, 2010). According to Table 4, the surface energy composed to polar and dispersive components; no significant changes were observed in the surface energy of PLA samples due to the addition of BHA, BHT and PG. However, TBHQ slightly decreased the PLA surface energy by reducing both dispersive and polar components. The surface energy of a polymer will influence its adhesive properties, and it is also important for processes such as printing and heat sealing.

3.7. XPS

XPS analysis was performed to examine the energy changes and chemical binding states of the polymer surfaces. Fig. 6 shows the C 1s and O 1s XPS spectra of the PLA film. The C and O peaks decomposed according to a model for biochemical compounds (Gerin, Dengis, & Rouxhet, 1995). The C 1s peak decomposed in three distinct peaks: a peak at 284.7 eV due to carbon atoms only bound to carbon and hydrogen (C–(C, H)), a second component was observed at 286.8 eV, attributed to carbon singly bound to oxygen (C–O) and the third at 288.8 eV due to carboxyl functions (C=O). The O 1s peak decomposed in two peaks attributed to the O=C, O–H at 531.3 eV and O–C at 532.7 eV. Table 5 presents the atomic composition of the PLA films before and after addition of antioxidants. SPAs had no significant effect on PLA surface atomic composition. As our XPS signal

Table 4
Contact angle and surface energy of PLA films containing 1% SPAs.

	Contact angle (°)	Surface energy (mJ/m ²)	Polar component (mJ/m ²)	Dispersive component (mJ/m ²)
PLA–BHA	77.2 ± 0.3 ^a	37.8 ± 1.4 ^a	5.1 ± 0.8 ^{ab}	32.7 ± 1.2 ^a
PLA–BHT	77.8 ± 1.4 ^a	37.1 ± 1.3 ^a	4.7 ± 0.6 ^a	32.4 ± 1.4 ^{ac}
PLA–PG	77.4 ± 1.1 ^a	35.4 ± 1.0 ^{ab}	6.1 ± 0.4 ^b	29.3 ± 0.8 ^b
PLA–TBHQ	78.4 ± 1.6 ^a	34.6 ± 0.9 ^b	6.1 ± 0.4 ^{bc}	28.6 ± 1.0 ^b
PLA	75.3 ± 1.6 ^a	36.3 ± 1.2 ^a	6.9 ± 0.5 ^{bc}	29.4 ± 1.6 ^{bc}

The means of three replicates plus or minus the standard deviation are shown. Significant difference ($P \leq 0.05$) is represented by different alphabetic letters.

Table 5
Atomic compositions and concentration of the different chemical bonds of PLA films containing 1% SPAs.

	O 1s	C 1s	Si 2p	C 1s C–(C, H)	C 1s C–O	C 1s O–C=O	O 1s O=C–O–H	O 1s O–C	C/O
PLA recto	36.82	62.21	0.97	38.57	30.58	30.85	44.34	55.66	1.68
PLA verso	37.70	62.30	–	35.37	32.63	32.00	43.53	56.47	1.65
PLA–BHA recto	36.16	62.93	0.91	38.34	30.64	31.01	44.81	55.19	1.74
PLA–BHA verso	36.97	63.03	–	36.99	31.36	31.65	42.57	57.43	1.70
PLA–BHT recto	37.33	62.67	–	35.26	32.58	32.16	42.70	57.30	1.67
PLA–BHT verso	36.48	63.52	–	35.49	32.40	32.11	43.24	56.76	1.74
PLA–PG recto	36.99	62.07	0.94	37.87	31.09	31.04	45.82	54.18	1.67
PLA–PG verso	36.52	63.48	–	37.32	31.12	31.56	43.07	56.93	1.73
PLA–TBHQ recto	35.93	62.70	1.37	40.25	29.74	30.01	45.58	54.42	1.74
PLA–TBHQ verso	36.06	63.94	–	37.41	31.52	31.07	44.77	55.23	1.77

All the tests were carried out in duplicate, Standard deviations are not shown.

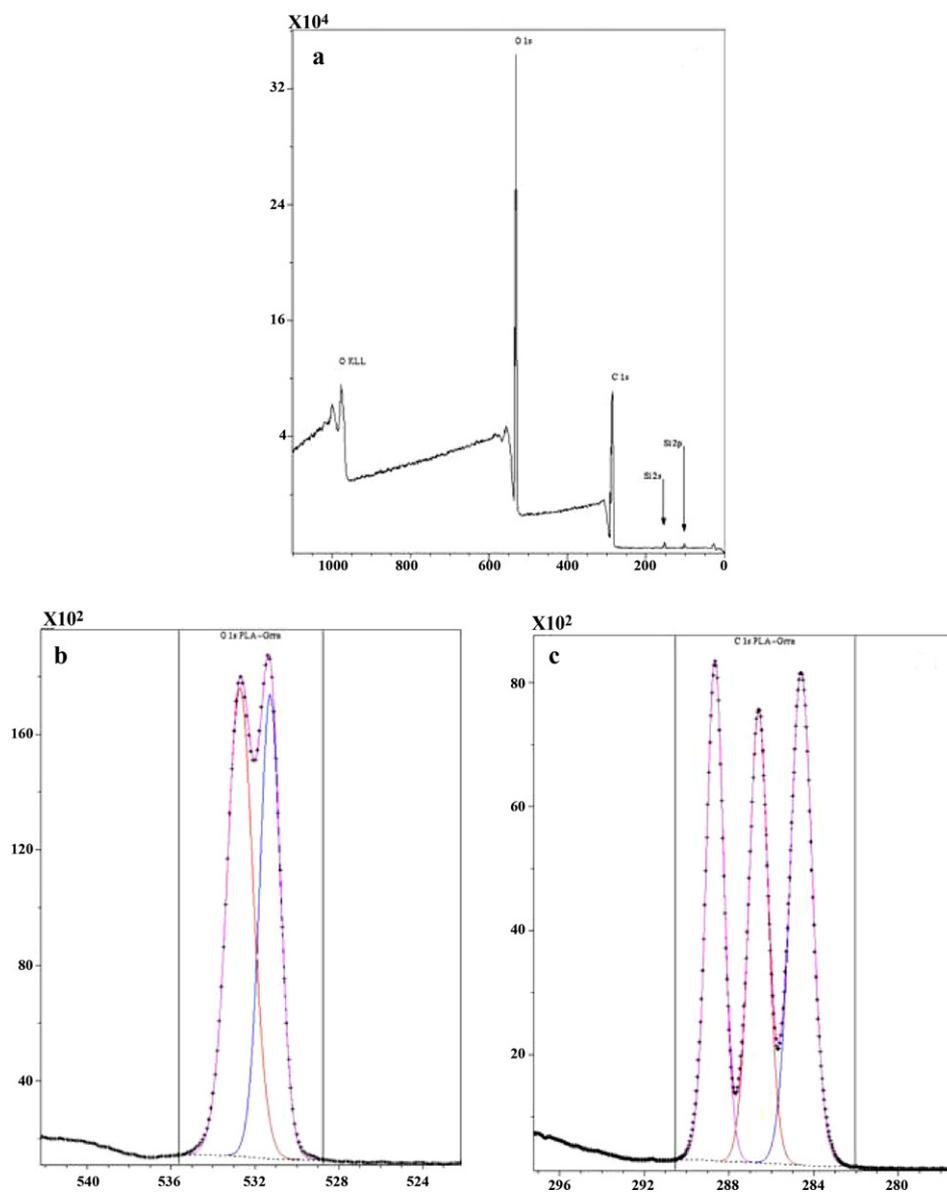


Fig. 6. XPS spectra obtained for PLA, a survey scan (a); narrow spectra of O 1s (b) narrow spectra of C 1s (c) levels. These peaks have been decomposed (see details in Section 3.6).

depth penetration was maximum 5 nm, it can be concluded that SPAs did not participate on extreme surface layer and distributed more in polymer matrix.

3.8. AFM results

Recently, tapping mode AFM has been used to investigate the surface structure and interactive force of nanostructured films (Jeong, Choi, Bae, Zentner, & Kim, 1999) and for instance to visualize crystalline morphology of poly(L-lactic acid) thin films (Li, Nie, Deng, Chen, & Ji, 2009). Therefore, in this study Intermittent Contact AFM (IC-AFM) was performed to get the better topographic images. AFM height images of PLA and PLA containing 1% SPAs thin films are displayed in Fig. 7. These images show that the surface microstructures of the films are drastically affected by adding the antioxidants.

PLA thin films (e) presented a homogenous smooth morphology with $R_a = 1.67$ nm and $R_q = 2.14$ nm. PLA–BHT (a) presents also

a homogeneous microstructure which can be associated with well-ordering microfibrils. The surface roughness increased compared with PLA thin films ($R_a = 8.24$ nm and $R_q = 10.4$ nm).

Adding 1% of TBHQ induced fragmented microfibrils surface microstructure. Grain-like particles appeared with some remained rodlet shaped. Compared with PLA thin films, PLA–TBHQ thin films had a near similar roughness in order of 2.11 nm and 2.75 nm for R_a and R_q , respectively.

By adding 1% of BHA to PLA film, the roughness parameters, R_a and R_q , are decreased with 0.673 nm and 0.855 nm, respectively. It can be observed a drastically change in microstructure surface compared to PLA films containing BHT and TBHQ antioxidants. PLA–BHA surface presented two micro-domains certainly associated with a difference of mechanical properties, one like PLA surface microstructure and the other one like orange skin.

By adding 1% of PG, the surface microstructure varied significantly compared to other surfaces where ellipsoidal structures

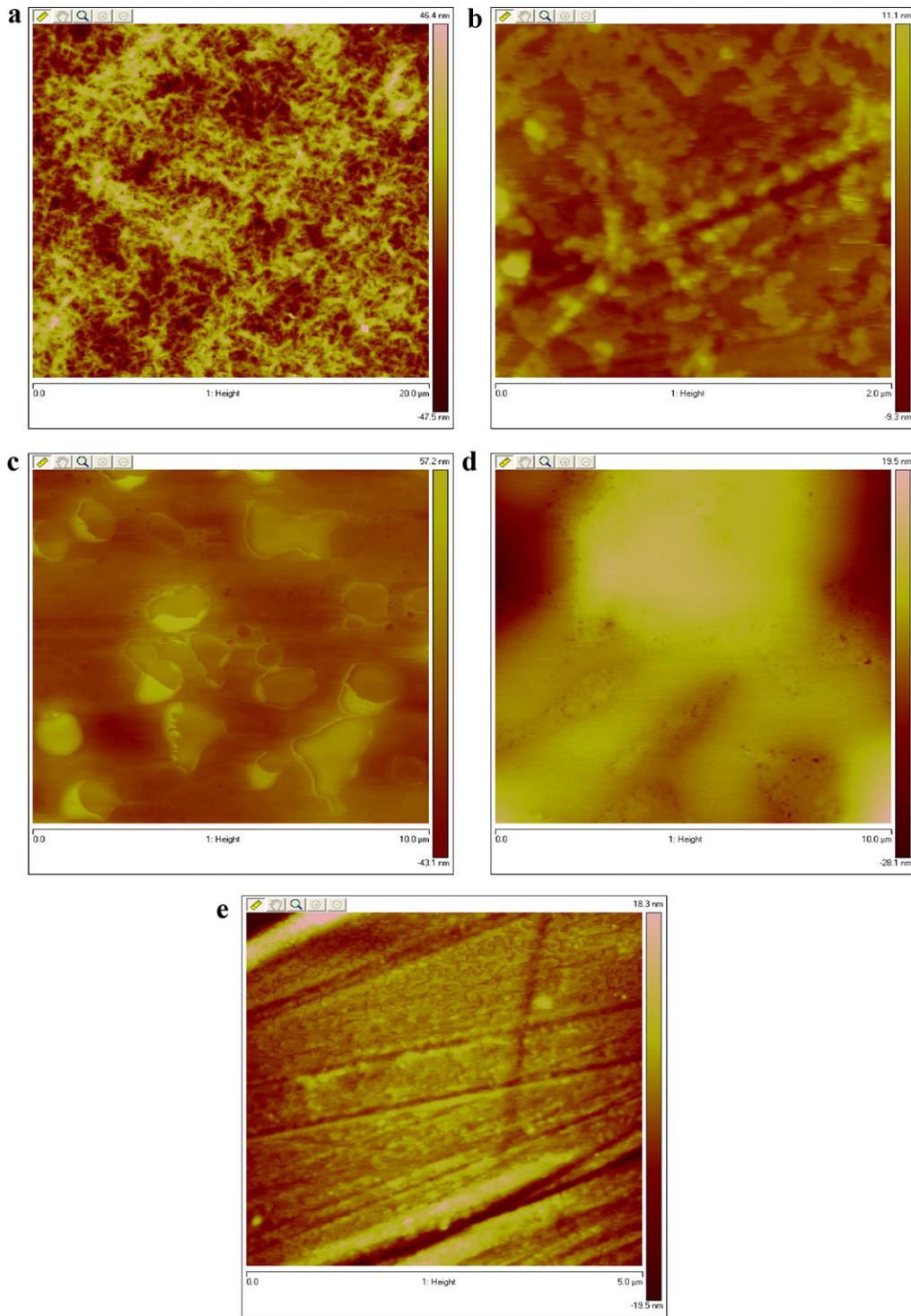


Fig. 7. AFM images of thin films, PLA–BHT (a), PLA–TBHQ (b), PLA–PG (c), PLA–BHA (d), and PLA (e).

with a flat center with a thickness in the order of 20 nm and axes $a \approx 1.3 \mu\text{m}$ and $b \approx 2 \mu\text{m}$ can be seen. It can be supposed that this organization could be attributed to atomic structure of PG antioxidants. The hypothesis should be confirmed in other work using for instance HarmoniX AFM mode (Hartman et al., 2010). As a matter of fact, the roughness of PLA–PG increased in comparing to PLA ($R_a = 1.67 \text{ nm}$ and $R_q = 2.14 \text{ nm}$).

In term of roughness parameters (R_a and R_q), we can classify the surface morphology following to this order: PLA–BHT > PLA–PG > PLA–TBHQ > PLA > PLA–BHA.

4. Conclusion

In this study, it was shown that addition of 1% of SPAs to PLA film by casting method results in changing of some physical and mechanical characteristics including 26% reduction in PLA crystallinity by all SPAs. Young's modulus and tensile strength of PLA film were slightly affected by BHA, PG and TBHQ. WVP of PLA was not changed by 1% addition of SPAs but the oxygen permeability of PLA film was influenced just by BHT. It was concluded that open chemical structure of BHT and its higher

molar volume can be considered as influencing factors. XPS results showed that two different faces of PLA films containing 1% of SPAs are not exactly the same and this suggested that SPAs are not uniformly distributed in film thickness because of partial antioxidant precipitation during polymer solution drying. Some microscopic methods such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are needed to investigate SPAs distribution in PLA matrix prepared by solvent-casting method. PLA film microstructures were significantly changed by addition 1% of SPAs; in term of roughness parameters (R_a and R_q), we can classify the surface morphology following this order: PLA-BHT > PLA-PG > PLA-TBHQ > PLA > PLA-BHA. In one hand, SPAs globally had not a significant modification action on PLA film properties and in the other hand their uses for creating active PLA packaging is technically safe.

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Chapitre 3.2

Propriétés Structurales, Mécaniques et Barrières du Film d'Acide Poly Lactique-Antioxydantes

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III. Résultats et Discussion

Chapitre 3.2: Propriétés Structurales, Mécaniques et Barrières du Film ...

Résumé

Le palmitate d'ascorbyle (AP) et l' α -tocophérol (AT) ont été ajoutés à des films d'acide polylactique (PLA) pour étudier leurs effets sur les propriétés physiques, structurales, mécaniques et barrière des films. Des cristaux d'AP sont apparus à la surface du PLA, après évaporation du solvant et ont changé la transparence du PLA. L'AP a diminué l'angle de contact du PLA du côté recto et augmenté la polarité et la mouillabilité du film d'APL. La spectroscopie X de photoélectrons a montré que l'AT a augmenté la teneur en C-(C, H), O=C et liaisons OH au verso, mais a diminué la teneur en liaisons CO et OC=O. La microscopie à force atomique a confirmé que la microstructure de surface du film de PLA a été considérablement influencée par l'addition d'antioxydants du point de vue des paramètres de rugosité (Ra et Rq). L'AT a diminué la température de la cristallisation de façon significative jusqu'à 74,1°C en raison de son effet plastifiant. Les AT et AP ont réduit le module d'Young et résistance à la traction des films du PLA. La perméabilité à la vapeur d'eau des films n'était pas significativement influencée par les AP et AT. L'AP n'est pas recommandé pour une utilisation dans le PLA afin d'obtenir un emballage actif.



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Structural, mechanical and barrier properties of active PLA–antioxidant films

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ABSTRACT

Ascorbyl palmitate (AP) and α -tocopherol (AT) were added to poly-(lactic acid) (PLA) film to investigate their effects on PLA's physical, structural, mechanical and barrier properties. AP crystals appeared on the PLA surface after solvent evaporation and changed PLA transparency. AP decreased the PLA contact angle on the recto side and increased film polarity and wettability. X-ray photoelectron spectroscopy showed that AT increased the concentration of C–(C,H), O=C and O–H bonds on the verso side, but decreased the concentration of C–O and O–C=O bonds. Atomic force microscopy confirmed that the PLA surface microstructure was drastically influenced by the addition of antioxidants in terms of roughness parameters (R_a and R_q). AT significantly decreased the film crystallization temperature down to 74.1 °C due to its plasticizing effect. Both AT and AP reduced PLA Young's modulus and tensile strength. PLA water vapor permeability was not significantly influenced by AP and AT. AP is not recommended to be used in PLA active packaging.

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1. Introduction

Free radicals have been found to be responsible for lipid oxidation, and hundreds of natural and synthetic compounds have been evaluated for their efficiency as radical scavengers or for their other inhibitory effects. Among synthetic antioxidants, only four are widely used in foods, namely butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) (Wanasundara and Shahidi, 2005). Recently, however, consumer demand for more natural and functional foods has increased because of their positive health benefits and the potential negative health effects of synthetic antioxidants.

The tocopherols are mainly present in oilseeds, oils, meats and the green parts of higher plants, whereas the tocotrienols are mostly found in the germ and bran fraction of certain seeds and cereals. The most abundant natural antioxidants in vegetable oils are the α - and γ -tocopherols, and the most important commercial compounds are α -, γ - and mixed tocopherols. The first two are commonly synthesized, while the latter is a by-product of vegetable oil processing. The antioxidant effects of tocopherols and tocotrienols are due to their ability to donate their phenolic hydrogen to

lipid free radicals and retard the autocatalytic lipid peroxidation processes. Alpha-tocopherol (AT) has the highest in vivo antioxidant activity for higher animals and humans, but the efficiency of tocopherols differs in different oxidation conditions (Seppanen et al., 2010).

Ascorbyl palmitate (AP) is a fat-soluble ester of palmitic acid and ascorbic acid, and could be used in oils or fatty foods. AP is a substance that is generally recognized as safe with no specific limitations or restrictions. Ingestion of this antioxidant would pose no health hazards because metabolic breakdown yields ascorbic acid and palmitic acid, both normal metabolites; furthermore it has been shown to be able to regenerate other antioxidants such as tocopherols (Lee et al., 1999; Karabulut, 2010).

Recently, the modification effects of natural antioxidants on polymers, especially biopolymers, have been investigated. Han and Krochta (2007) studied oxygen permeability parameters including the oxygen solubility and diffusivity of two differently-made whey protein isolate (WPI) coatings (powder blended and ethanol solvent mixing) containing 10% of AT and AP. They showed that AP and AT addition increased the oxygen diffusivity approximately 10-fold, and oxygen solubility decreased 10-fold. As a result, the permeability of antioxidant-incorporated films was not enhanced compared to control WPI films. Lopez-Rubio and Lagaron (2010) described the UV stability and mechanical properties of biopolyesters such as poly-(lactic acid) (PLA), polycaprolac-

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ton (PCL) and polyhydroxybutyrate-co-valerate (PHBV) containing β -carotene. They showed a plasticizing effect of β -carotene resulting in a significant increase in the deformation at break and lower Young's modulus. The results confirmed that even though UV radiation degraded β -carotene in polyesters, their mechanical properties remained more stable than those of the pure polymers. The authors therefore suggested β -carotene as a natural additive to increase the UV stability of biopolyesters.

Characterization of extruded PLA-AT was accomplished by Byun et al. (2010). They used polypropylene glycol and BHT as a plasticizer and an antioxidant for film extrusion processing, respectively. They concluded that adding AT to PLA increases its antioxidant activity, oxygen permeability and elongation at break, but decreases film water permeability. According to the authors' conclusion, PLA clarity, which was 20 times less than in pure PLA, is the sole limitation of AT application for PLA active packaging; they did not consider the increase in oxygen permeability as a negative factor for PLA-AT. Gonçalves et al. (2011) studied the addition effect of 2.2% and 4.4% of AT on PLA mechanical, thermal and gas barrier properties. They found no significant changes in the mechanical and thermal properties, but concluded that AT addition increases the oxygen sorption to PLA film.

PLA has been adopted as a biodegradable thermoplastic polyester in packaging, construction, automotive, appliance and medical applications. However, its weaknesses include poor gas-barrier properties, low toughness and ductility, poor thermal stability and high cost, so that many research studies have focused on overcoming these limitations by different polymer modifying methods (Dorgan et al., 2000; Harada et al., 2007). Modifiers such as citrate esters, triacetine, tributyl citrate, oligomeric malonate esteramides, 4,4-methylene diphenyl diisocyanate, polyglycerol esters, polyethylene glycol, acetyl triethyl citrate, talc, bifunctional cyclic ester, and poly (1,3-butylene adipate) have been investigated for PLA improvement (Jamshidian et al., 2010).

Apart from the potential modifying effects of antioxidants, antioxidant-active packaging is an important category of active packaging and a promising technique for extending food shelf-life. Several polymer films have been impregnated with different antioxidants, and the antioxidants' migrations in real foods and food stimulants have been studied (Schwope et al., 1987; Wessling et al., 1999, 2001; Granda-Restrepo et al., 2009). In most studies, the important objectives were the rates, effects and mechanisms of antioxidant release.

Polymer's mechanical, physicochemical and barrier characteristics affect its technical properties and stability in various conditions, whereas its structural properties can influence the rate and amount at which active agents migrate. The first group of characteristics is routinely examined when using modifying compounds, but the structural properties are rarely studied precisely. The addition of active compounds may affect the structure and other physical and mechanical characteristics important to the technological and functional aspects of packaging materials, so an active packaging without the desired properties will be useless. However, the authors believe that the first step in creating a successful active packaging is to identify the effects and interactions between the active compound and the polymer structure. The active agent may have some potential negative effects on the film's functional characteristics; for example, it may cause higher oxygen and water vapor transfer, or weaken the film's mechanical properties.

Atomic force microscopy (AFM) technique can provide precise information about morphological changes made by modifiers that can affect their release into foodstuffs (Kikkawa et al., 2004; Sanchez-Garcia et al., 2008). Surface energy and contact angle provide information concerning the wettability and polarity of the polymer. X-ray photoelectron spectroscopy (XPS) determines the chemical composition of the surface layer and characterizes possi-

ble chemical bonding between the modifying agent and the polymer (Kiss et al., 2002).

In this study, the addition effects of two antioxidants, AT and AP, on PLA film made by the solvent-casting method were investigated. The addition of these active compounds may affect the physical, structural, barrier and mechanical properties of PLA film, which are important in respect of the technological and functional aspects of packaging materials. The changes in the thermal and mechanical properties of the obtained materials were addressed using differential scanning calorimetry (DSC) and universal testing machine, respectively. The microstructural surface characterizations of PLA film were analyzed by contact angle, surface energy, XPS and AFM.

2. Materials and methods

2.1. Materials

Poly-(lactic acid) (PLA), 2002D, was purchased in pellet form from Natureworks® Co., Minnetonka (USA); (+)- α -tocopherol obtained from vegetable oil (1000 IUg^{-1}), ascorbyl palmitate (6-O-Palmitoyl-L-ascorbic acid), magnesium nitrate and phosphorus pentoxide from Sigma-Aldrich (France); deuterated chloroform ($99.8\% \text{ CDCl}_3$) from Euriso-Top (France), HPLC-grade chloroform and potassium nitrate from VWR international (France).

2.2. Film preparation method

At first a solution of 3.5% w/w of PLA was prepared in chloroform, and afterwards 1% w/w of AP and 2% w/w of AT (2% was used because of supposed higher sensibility of AT to light and temperature) based on PLA dry matter were added to the solution and mixed for 1 h at ambient temperature for complete distribution of antioxidants in the polymer matrix. Because AP was not completely soluble in chloroform, some droplets of pure ethanol were added. Then 20 g of polymer solution was cast in Teflon $90 \times 110 \text{ mm}$ Petri dishes (Welch, USA), and the extra chloroform was allowed to evaporate in a hood in a dark place over seven days at 25°C . The Petri dishes were kept in a hermetic container containing P_2O_5 powder until the time of each analysis. The final film thickness was $100 \pm 5 \mu\text{m}$ measured (at least eight points) by a mechanical micrometer (Messmer, UK) according to ASTM D 374-99.

2.3. Determination of α -tocopherol and ascorbyl palmitate in PLA films

The amounts of antioxidants in PLA films after drying were measured by nuclear magnetic resonance spectroscopy (NMR). ^1H NMR chemical shifts in ppm were collected at 600.13 MHz on an Avance III 600 spectrometer (Bruker, Germany) at 298°K . PLA films were dissolved in deuterated chloroform and antioxidants were quantified by comparing the phenolic group peak area of each antioxidant in the sample with phenolic peak area of pure antioxidant solutions (1, 5 and 10 mM). Standard precautions regarding quantization in NMR were taken, including identical spectrometer settings and long enough repetition time between two consecutive scans in comparison with relaxation times. All tests were accomplished in triplicate.

2.4. Surface characterization

2.4.1. Film morphological structure

PLA film surface structures were observed at ambient temperature using an Olympus (Provis AX70, Japan) polarizing optical microscope equipped with a digital video camera system.

2.4.2. Measurement of contact angle

The contact angle of the films was obtained at 20 °C using a commercial Digidrop meter (GBX Scientific Instruments, France) equipped with a charge-coupled device (CCD) camera 2/3" and a Nikon 60 mm macro lens. This apparatus consists of software named Windrop⁺⁺, which precisely determined the drop size of equilibrated liquid placed on the sample surface. The contact angle was measured by randomly adding 10 drops of ultra-pure liquids (2 µl) on the surface of a film disk (80 mm in diameter). Using Windrop⁺⁺ software provided with an instrument, the measurements of the static contact angle values were fully automated.

2.4.3. Measurement of surface energy

The total surface energy of PLA films was determined graphically by the Owens–Wendt method, which is usually used for solids with low surface energy such as polymers. The Owens–Wendt theory divides the surface energy into two components: surface energy due to dispersive interactions and surface energy due to polar interactions. Owens and Wendt proved that the total surface energy of a solid, γ_s , can be expressed as the sum of contributions from dispersion γ_s^d and polar γ_s^p force components ($\gamma_s = \gamma_s^d + \gamma_s^p$). The Owens–Wendt equation (Eq. 1) applies the contact angle, θ , data of polar and non-polar liquids with known dispersion γ_L^d and polar γ_L^p parts of their surface energy (Michalski et al., 1998, 1999).

$$(\gamma_L(1 + \cos\theta))/(2(\gamma_L^d)^{1/2}) = ((\gamma_s^p\gamma_L^p)^{1/2}/(\gamma_L^d)^{1/2}) + (\gamma_s^d)^{1/2} \quad (1)$$

where γ_L is the liquid surface tension.

By plotting $(\gamma_L(1 + \cos\theta))/(2(\gamma_L^d)^{1/2})$ versus $(\gamma_L^p\gamma_L^d)^{1/2}$, the polar and dispersive components of the solid's surface energy were determined by the slope and intercept of the resulting graph, respectively. For this measurement, four liquids including water, glycerol, ethylene glycol and diiodomethane tested for each PLA film at 23 °C and 50% RH.

2.4.4. X-ray photoelectron spectroscopy (XPS)

The XPS analyses were carried out with a Kratos Axis Ultra spectrometer (Kratos Analytical, Manchester, UK) using a monochromatic Al K α source. All spectra were recorded at a 90° take-off angle and the analyzed area was about 700 × 300 µm. Survey spectra were recorded with 1.0 eV step and 160 eV analyzer pass energy, and the high resolution regions with 0.1 eV step and 20 eV pass energy, except for the carbon, which was recorded with 0.05 eV step. In both cases, the hybrid lens mode was employed (magnetic and electrostatic). During the data acquisition the Kratos charge neutralizer system was used on all specimens with the following settings: filament current 2A, charge balances 3.5 V, filament bias 1.0V and magnetic lens trim coil 0.34A. The C–(C,H) carbon was set to 284.60 eV and therefore used as an internal energy reference. Spectra were analyzed using the Vision software from Kratos (Vision 2.2.6). A Shirley base line was used for the subtraction of the background from each peak. Quantification was performed using the photoemission cross-sections and transmission coefficients given in the Vision package.

2.4.5. Atomic force microscopy analysis (AFM)

PLA film's surface morphology was analyzed by using a D3100 AFM equipped with a Nanoscope 5 electronic from the manufacturer Veeco. The images were recorded at ambient conditions (20 °C and 30% RH) and in soft intermittent contact mode (IC-AFM or TappingTM AFM). Tap150 tapping mode cantilevers (Veeco model No. MPP-12100), with a typical spring constant of about 5 N/m and a resonance frequency around 165 kHz, were used for scanning. Tapping force was controlled by the ratio between set point amplitude (A_{sp}) and free-air amplitude (A_0). The scan rate

was adjusted in the range of 0.5–1 Hz depending on the image quality. Each scan line contains 512 pixels and a whole image is composed of 512 scan lines. For acquisition of the surface morphology, amplitude error, phase and height images were recorded on several areas of the film surface. Only the representative height images are presented here for discussion with a scan size of 50 µm² for PLA-AP and 5 µm² for PLA-AT and pure PLA.

All offline image flattening and analyses were conducted in the software environment provided by the AFM manufacturer. Using the equipment's software, the statistical parameters related to sample roughness were estimated according to ASME (1995), including:

Average roughness (R_a): the average of the absolute value of height deviations from mean surface;

Root mean square roughness (R_q): the root means square average of height deviations from the mean date plane;

Skewness (S_k): the measure of asymmetry of data or, more precisely, the lack of symmetry;

Kurtosis (E_k): the measure of amplitude distribution or, more precisely, the measure of whether the data are peaked or flat relative to a normal distribution.

2.5. Thermal properties

Thermal analysis for measuring transition temperatures, including glass-transition temperature (T_g), melting temperature (T_m), enthalpies of fusion (ΔH_f) and crystallization (ΔH_c), was carried out with a differential scan calorimeter (NETZSCH, 204F1 Phonex, Germany). Specimens weighing approximately 10 mg were sealed in an aluminum pan and heated under protective nitrogen (40 mL min⁻¹) at the rate of 10 °C min⁻¹ from 5 to 210 °C, then cooled to 5 °C and reheated to 210 °C with the same cooling-heating rates.

T_g , T_c and T_m were determined from the obtained thermogram. The degree of crystallinity was evaluated according to Eq. (2):

$$\% \text{ Crystallinity of PLA} = 100 \times [(\Delta H_m - \Delta H_c)/\Delta H_f] \quad (2)$$

where: ΔH_m = Enthalpy of fusion; ΔH_c = Enthalpy of crystallization; ΔH_f = Enthalpy of fusion of a wholly crystalline PLA (93.6 J g⁻¹) (Arnoult et al., 2007).

2.6. Mechanical properties

A Lloyd instruments universal testing machine (AMETEK, LRX, UK) was used to determine different mechanical properties including tensile strength, Young's modulus and elongation at break according to ASTM D 882-02. Before testing, all samples were equilibrated for 48 h at 50 ± 2% relative humidity (RH) in a container using magnesium nitrate saturated solution at 20 ± 1 °C. Equilibrated film specimens were mounted in the film-extending grips of the testing machine and stretched at a rate of 25 mm min⁻¹ until breaking. The relative humidity and temperature of the testing environment were held at 52 ± 2% RH and 20 ± 2 °C, respectively.

2.7. Water vapor permeability (WVP) measurement

WVP was measured by gravimetric method according to ASTM E 96-00. Film specimens, with surface equal 25 cm², were placed between two Teflon rings in the cap of the permeability cell. To reach a gradient of 90% RH at both sides of the sample, the cells were filled with CaCl₂ (dried at 200 °C and cooled before use) as a desiccant. Cup cells were placed in a conditioned chamber (Climacell, MMM Medcenter Einrichtungen GmbH, Czech Republic) at a controlled temperature of 38 ± 1 °C and RH of 90 ± 1%

and weighed twice a day at regular intervals by a balance with 0.001 g precision. Continuous air circulation through the chamber maintained uniform conditions at all test locations. Weight gain from each cup was measured for seven days and a curve of cumulative mass gain was obtained as a function of time. Rate of water vapor transmission (WVT, $\text{g s}^{-1} \text{m}^{-2}$) was determined from the slope obtained from the regression analysis of weight gain data as a function of time, once the steady state was reached. Permeance ($\text{g m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$) was obtained by Equation (3).

$$\text{Permeance} = (\Delta m/\Delta t)/A \cdot \Delta p \quad (3)$$

where: $\Delta m/\Delta t$: slope of the linear part of the kinetic curve (g s^{-1}); A: Film surface (m^2); Δp : Vapor pressure difference at both sides of the film (Pa).

Water vapor permeability or WVP ($\text{kg m m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$) was calculated by Eq. (4):

$$\text{WVP} = \text{Permeance} \times e \quad (4)$$

where e is film thickness (m).

2.8. Statistical analysis

The data were analyzed by analysis of variance (ANOVA) using StatGraphics (version 16, StatPoint Technologies, Inc., Warrenton, VA, USA) and differences among mean values were processed by the Student–Newman–Keuls multiple range test. Multiple comparisons were performed through 95% least significant difference intervals ($P \leq 0.05$).

3. Results and discussion

3.1. Amounts of α -tocopherol and ascorbyl palmitate in PLA film

During the preparation of samples a loss of bioactive compounds was observed. From NMR spectroscopy (data not shown) the recovery of AP and AT was evaluated. PLA films loaded with 1% (w/w) AP and 2% AT yielded a recovery of 65% and 90%, respectively. These losses can be attributed to antioxidant degradation and probably the destructive effects of light. However, AT degradation did not occur as expected and, contradictory to our assumption, AP degradation was much more than estimated. The greater quantity of AT added in this study was based on the 34% loss of AT reported in poly(lactide-co-glycolide) (PLGA) films (Van Aardt et al., 2007). The volatility of antioxidants is desired for dried food applications because of their rapid migration via evaporation from the polymer surface to target food (Wessling et al., 2001).

3.2. Morphological structure

PLA film had a different appearance depending on whether it contained AT or AP: PLA-AT was transparent, like pure PLA, but PLA-AP had a rough surface on the recto side (the face in contact with air) and a flat surface on the verso side (the face in contact with Teflon). So it was supposed that AP recrystallized from polymer solution during chloroform evaporation. As the density of AP (bulk density = 240 kg m^{-3}) is less than that of PLA (specific gravity = 1.4), AP crystals appeared on the PLA surface. To prove the AP recrystallization phenomenon, different concentrations of AP (0.1%, 0.3%, 0.6% and 1% w/w) were added to PLA solutions and their film surfaces were observed under a polarized optical microscope (Fig. 1). As seen, the number and size of the AP crystals increased as the AP concentrations were augmented, confirming the AP crystallization phenomenon. The AP crystallization process was also time-dependent; the film drying velocities (at 20 °C and 50 °C) had a significant effect on the size of the AP crystals. PLA film

containing 1% AP dried at 20 °C had a larger AP crystal size (500–10000 μm) than PLA-AP film dried at 50 °C (5–15 μm). AT did not change the appearance of PLA, but its crystals were visible in the polymer matrix under a polarized optical microscope (Fig. 2).

3.3. Contact angle and surface energy

The water contact angle, surface energy, polar component and dispersion component of the PLA films are presented in Table 1. The surface energy of PLA at two surfaces (recto, the surface in contact with air, and verso, the surface in contact with Teflon) was increased unequally by the addition of AT. The verso surface of PLA-AT had a higher surface energy than the recto, and also than pure PLA; this change was observed in both polar and dispersion components as well. AT has a hydrophobic nature, and the different surface energies of PLA-AT surfaces may be caused by the heterogeneous distribution of AT in these surfaces. AP decreased the PLA contact angle at the recto side and increased film polarity and wettability. Conversely, AP as a hydrophobic molecule was expected to increase the contact angle; this reduction can be attributed to the non-uniform structure of the PLA surface caused by AP crystals. The PLA surface energy was not changed by AP, but the polar and dispersion components were significantly modified on the recto side.

3.4. XPS

XPS analysis was performed to investigate the chemical bonding states of polymer surfaces. As shown in Table 2, the atomic composition of the PLA films before and after adding the AP was not significantly changed. In contrast, by adding AT, the composition of PLA was changed on the verso side only, resulting in a different composition of the film on its two sides. According to a model for biochemical compounds, the C and O peaks were decomposed (Gerin et al., 1995). The C1s peak was decomposed in three peaks corresponding to C–(C,H), C–O and O–C=O functions. The O1s peak was decomposed in two peaks attributed to the O=C, O–H and O–C or H₂O.

Table 2 clearly shows that after AT incorporation, the concentration of C–(C,H), O=C and O–H bonds are increased on the verso side, while the concentration of C–O and O–C=O bonds are decreased. These chemical bonding changes can affect polymer surface energy; as can be seen in Table 1, the surface energy of PLA-AT on the verso side is higher than that of other PLA films, confirming this phenomena. From the above results, it can be concluded that the addition of AT changes the polarity of the PLA surface. The addition of AP slightly changed the surface chemical bonds, including C–(C,H), O–C=O, O=C and O–H bonds which could be produced by AP crystals. PLA-AT on the recto side and PLA-AP on the verso side had the same chemical composition as PLA.

3.5. AFM results

Recently, tapping mode AFM has been used as a standard tool to investigate the surface structure and interactive force of nanostructured films (Jeong et al., 1999) and, for instance, to visualize the crystalline morphology of PLA thin films (Li et al., 2009). Therefore, in this study the IC-AFM was performed to get better topographic images. AFM height images of thin films studied are displayed in Fig. 3, showing that the surface microstructure of the film is drastically affected by adding the antioxidants. PLA thin films (Fig. 3c) present a homogenous smooth morphology with $R_a = 1.67 \text{ nm}$ and $R_q = 2.14 \text{ nm}$. PLA-AT surfaces (Fig. 3a) present a pseudo-regular arrangement of nano-dots. These microstructure

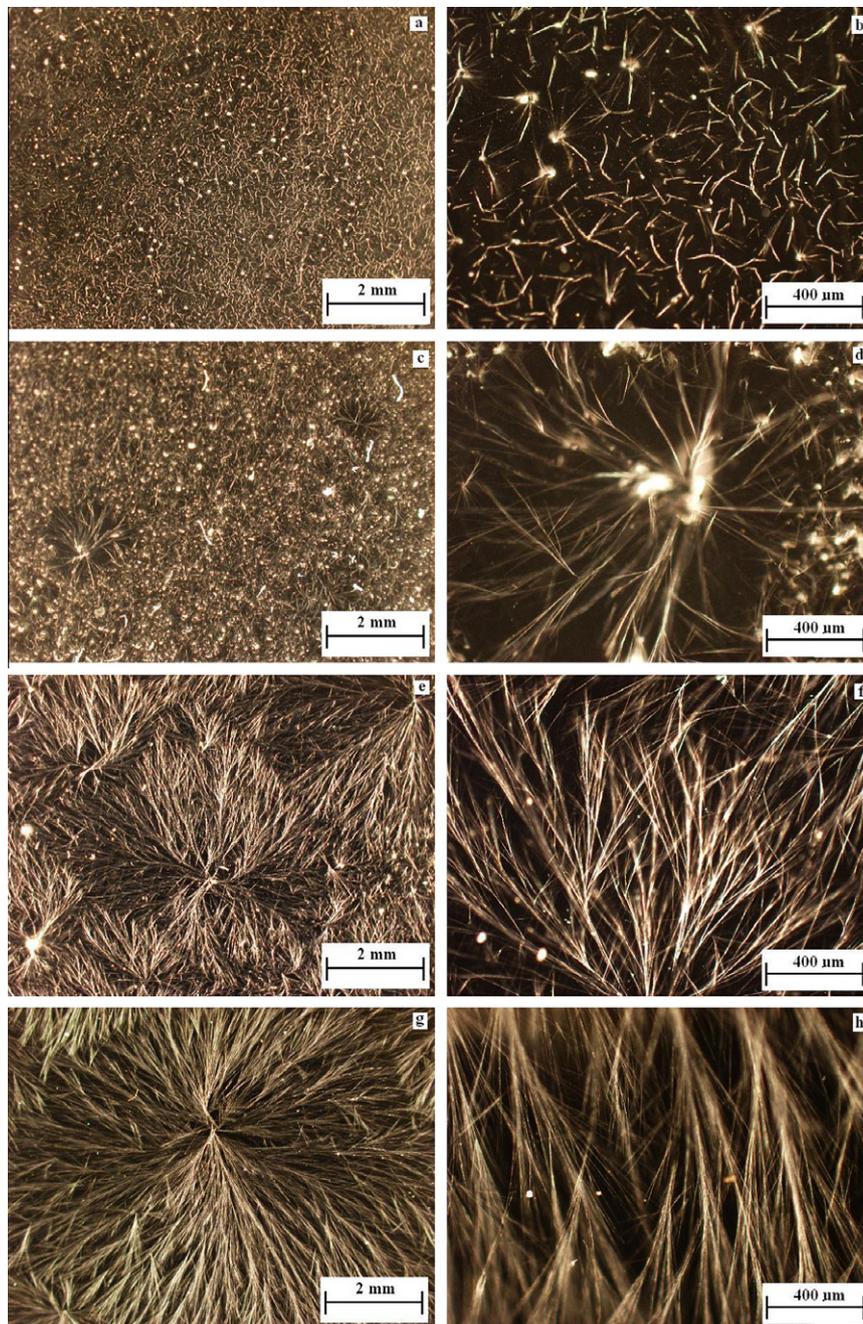


Fig. 1. AP crystals in PLA-AP films containing 0.1% (a, b), 0.3% (c, d), 0.6% (e, f) and 1% (g, h) AP, images were taken at ambient temperature.

surfaces are certainly associated with both mechanical properties and the particular structure of PLA-AT.

Compared to PLA thin films, PLA-AT thin films had a near similar roughness in order of 1.40 nm and 2.32 nm for R_a and R_q , respectively. As shown in Fig. 1, on adding the AP to PLA film, AP crystals appeared on the PLA surface and made a branching structure; the roughness parameters, R_a and R_q , were significantly increased with 7.36 and 8.8 nm outside the branch and 25.7 and 29.9 inside the branch.

AP made a drastic change to the PLA surface microstructure compared to AT or pure PLA thin films, whereas a layered structure can be observed in Fig. 3b. The 3D reconstruction of the surface and the transversal cut of PLA-AT and PLA-AP are presented in Fig. 4. The transversal cut of PLA-AT represents the small dots with a height in the order of 20 nm and diameter of around 0.2 μm . The

image of PLA-AP in this figure shows a scale of a thickness of around 20 nm.

In Fig. 4, it can be observed that AP has created a layered structure on the recto side of PLA-AP film. The core of this topographical approach then is the definition of the reference behavior. Thus, in order to define the effects of abrasion on topographical parameters precisely, it is necessary to follow the variation of complementary parameters, and especially the profile symmetry/height balance, via both E_k and S_k parameters and amplitude parameters including R_a and R_q . The roughness parameters for PLA-AP were not calculated because of its layered structure.

Compared to PLA thin films ($S_k = -0.15$ nm and $E_k = 6.1$ nm), PLA-AT thin films had an S_k and E_k in the order of 4.3 and 31 nm respectively. A negative skewness indicates a concentration of the material near the top of the profile signifying a tray-like

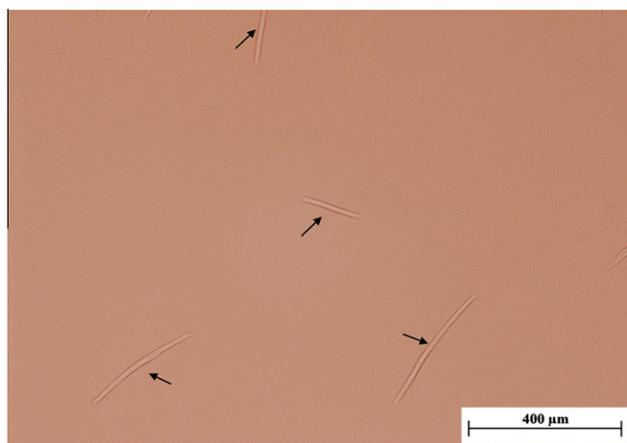


Fig. 2. PLA film containing 2% AT observed by polarized optical microscope taken at ambient temperature. The Arrows show the AT crystals.

surface. A Kurtosis >3 indicates sharp amplitude distribution, that is to say large, sharp peaks and valleys. By adding AP to PLA film, S_k and E_k were decreased to -0.35 and 2.2 nm outside the branch and with 0.24 and 2.1 inside the branch.

It was considered that there is a correlation between S_k and the surface energy of PLA films; as shown in Table 3, it can be seen that with increasing surface energy, the S_k parameter tends to increase, proving the correlation of two microscopic parameters for this kind of polymer.

3.6. Thermal properties

DSC thermographs of PLA films containing 1% AP, 2% AT and also pure PLA are depicted in Fig. 5, and their thermal properties have been shown in Table 4. The pure PLA exhibited a T_g around 60 °C and a melting temperature (T_m) around 150 °C. Calculated PLA crystallinity according to Equation 2 was 20.3% , and crystallization temperature (T_c) was around 95 °C.

The T_g was reduced to 58.2 °C with the addition of AT. The incorporation of active agents in polymer almost increases the free volume of polymer matrix and may change the polymer thermal

properties; it is well known, for example, that by addition of a plasticizer the T_g of polymer is decreased (Kulinski et al., 2006; Pillin et al., 2006; Lopez-Rubio and Lagaron, 2011). We therefore considered that these antioxidants acted as a pseudo plasticizer in the PLA matrix. The plasticizing influence of AT and AP is not noticeable in this study because of the small concentrations added. The reason for the lack of change in thermal properties brought about by AP can be attributed to some extent to its recrystallization on the PLA surface and thus its partial separation from the polymeric phase.

Addition of 2% AT to PLA significantly reduced the film's T_c to 74.1 °C. A decreasing and narrowing of T_c by the addition of plasticizers has been already reported. A phenomenon which can be associated with this effect is the reduced crystallization induction period due to the presence of AT crystals which acted as crystalline nuclei. Even though these nuclei may represent a small crystalline fraction in absolute terms, they will increase the crystallization rate upon heating. Besides, the reduction in crystallization temperature is consistent with the fact that PLA crystallizes with more ease at lower temperatures due to enhanced chain mobility provided by the AT (Martin and Avérous, 2001). Lopez-Rubio and Lagaron (2011) found that the addition of β -carotene to PLA increases the crystallinity of the polymer and also changes the type of melting profile from a single melting peak to a double event. They also observed a decrease in cold crystallization temperature in β -carotene-containing PLA film, and concluded this is because of the nucleating effect of β -carotene during film forming. Extruded PLA film containing 1% AT and 10% polyethylene glycol 400 (PEG 400) had a sharper crystallization peak, AT also diminished T_c to around 83 °C, but no change in melting temperature was observed (Byun et al., 2010).

AP and AT had a reducing influence (about 7%) on PLA crystallinity; it seems that AP and AT somehow hinder polymer chains' lateral rearrangements and hence crystallization in PLA. It was shown that fatty acid ester is a good plasticizer with a strong hindrance of the crystalline phase development (Jacobsen and Fritz, 1999). A similar effect was observed with cellulose fiber and β -carotene in PCL and PHBV respectively (Lopez-Rubio and Lagaron, 2010; Sanchez-Garcia et al., 2008). However, the decreasing effect of AT on PLA crystallinity contradicts the results of Gonçalves et al. (2011), which show a slight increase in PLA crystallinity with the

Table 1
Surface characteristics of PLA and PLA containing 1% AP and 2% AT.

	Contact angle ¹ (°)	Surface energy (mJ/m ²)	Polar component (mJ/m ²)	Dispersion component (mJ/m ²)
PLA-AT 2% recto	77.6 ± 1.4 ^a	38.6 ± 1.5 ^a	5.0 ± 0.8 ^a	33.6 ± 1.4 ^b
PLA-AT 2% verso	77.8 ± 1.6 ^a	44.3 ± 1.7 ^b	3.6 ± 0.6 ^a	40.6 ± 1.5 ^c
PLA-AP 1% recto	69.1 ± 1.9 ^b	35.6 ± 1.8 ^a	12.9 ± 0.6 ^c	22.7 ± 1.6 ^a
PLA-AP 1% verso	77.4 ± 1.9 ^a	35.1 ± 1.6 ^a	6.1 ± 0.7 ^a	28.9 ± 2.2 ^b
PLA (recto-verso)	75.3 ± 1.3 ^a	36.3 ± 1.1 ^a	6.9 ± 0.2 ^b	29.4 ± 1.3 ^b

1-Water contact angle.

The means of three replicates plus or minus the standard deviation are shown. Significant difference ($P \leq 0.05$) is represented by different alphabetic letters.

Table 2
Atomic compositions and concentrations of the different chemical bonds at PLA films surface.

	O 1s	C 1s	C1s C-(C,H)	C1s C-O	C1s O-C=O	O1s O=C, O-H	O1s O-C
PLA-AT, R ¹	36.2 ± 1.2 ^b	63.0 ± 1.4 ^a	38.8 ± 1.2 ^a	30.7 ± 1.2 ^b	30.5 ± 1.1 ^{bc}	46.1 ± 1.4 ^a	54.2 ± 1.2 ^b
PLA-AT, V ¹	10.2 ± 0.4 ^a	89.8 ± 2.1 ^b	84.0 ± 1.5 ^c	12.1 ± 0.6 ^a	3.9 ± 1.2 ^a	56.1 ± 1.2 ^b	43.9 ± 1.8 ^a
PLA-AP, R	34.3 ± 1.2 ^b	64.8 ± 1.3 ^a	44.2 ± 1.3 ^b	29.0 ± 1.1 ^b	26.8 ± 1.2 ^b	47.7 ± 1.7 ^a	53.7 ± 1.8 ^b
PLA-AP, V	36.6 ± 1.2 ^b	63.3 ± 1.5 ^a	36.4 ± 1.0 ^a	32.2 ± 1.2 ^b	31.4 ± 1.7 ^{bc}	44.3 ± 1.2 ^a	55.7 ± 1.3 ^b
PLA, R	36.8 ± 1.2 ^b	62.2 ± 1.3 ^a	38.6 ± 1.1 ^a	30.6 ± 1.2 ^b	30.8 ± 1.2 ^{bc}	44.3 ± 1.1 ^a	55.7 ± 1.2 ^b
PLA, V	37.7 ± 1.1 ^b	62.3 ± 1.5 ^a	37.4 ± 1.2 ^a	31.6 ± 1.2 ^b	32.0 ± 1.2 ^c	43.5 ± 1.2 ^a	56.5 ± 1.3 ^b

R = recto, V = verso.

The means of three replicates plus or minus the standard deviation are shown. Significant difference ($P \leq 0.05$) is represented by different alphabetic letters.

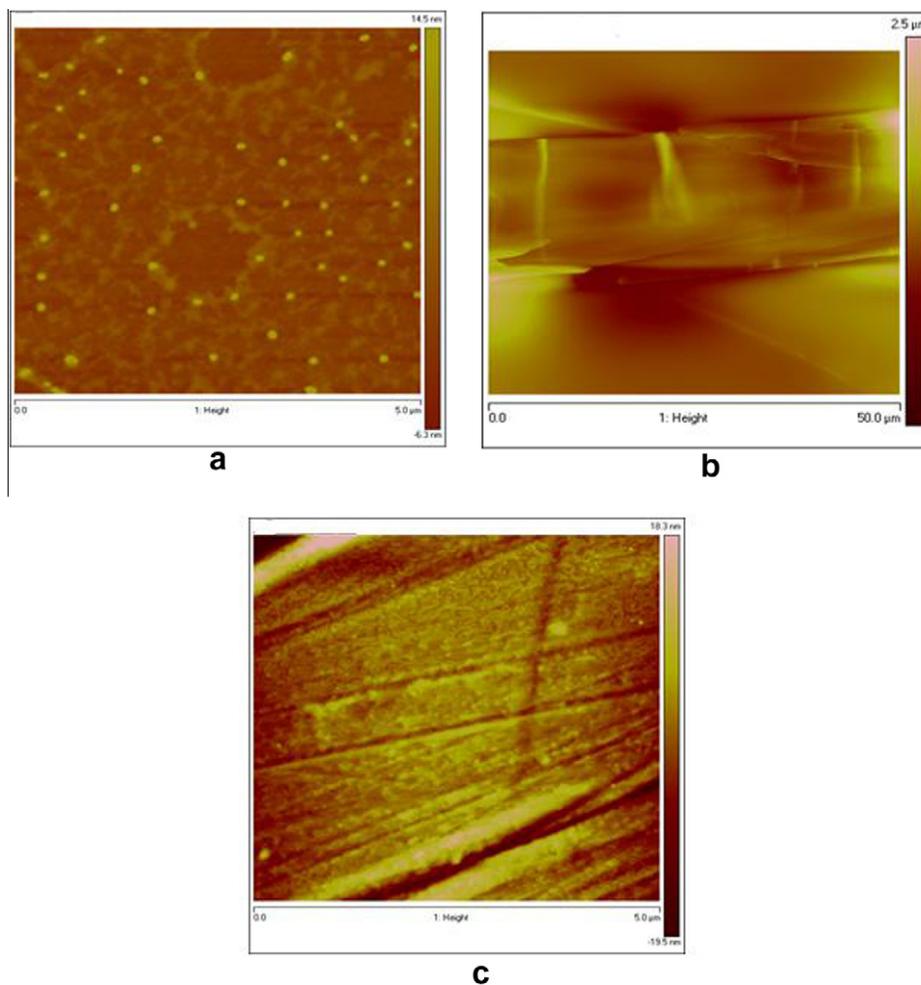


Fig. 3. AFM height images of (a) PLA-AT, (b) PLA-AP, and (c) PLA thin films. A larger scale for PLA-AP was selected for covering the big AP crystal.

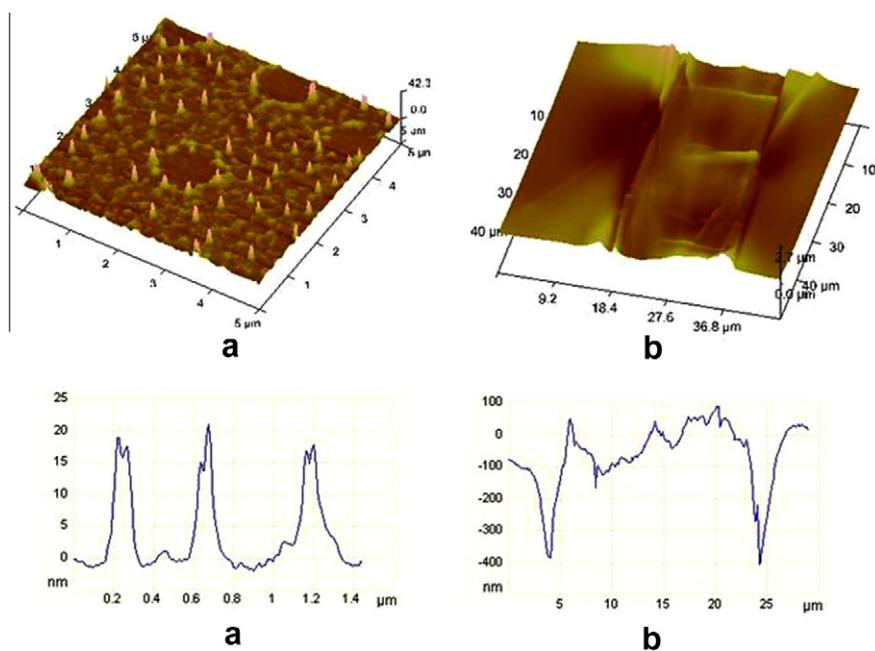


Fig. 4. AFM images for (a) PLA-AT and (b) PLA-AP thin films. The first row shows the 3D reconstruction of the surface and the second one the transversal cut. The larger scale was selected for PLA-AP because of big AP crystal size.

Table 3
Evolution of the surface energy in function of S_k .

	Surface energy (mJ/m ²)	S_k (nm)
PLA-AP, V	35.1 ± 1.6 ^a	-0.35 ± 0.02 ^a
PLA-AT, V	44.1 ± 1.7 ^b	4.29 ± 0.31 ^c
PLA	36.3 ± 1.1 ^a	-0.15 ± 0.01 ^b

addition of 4.4% AT to PLA. On the one hand, AP decreased PLA crystallinity and on the other, it caused AP surface crystallization, so it can be assumed that AP also acted to impair crystal development in PLA polymer matrix. Otherwise, plasticizers interfere with the arrangement of the polymer chains and the hydrogen bonding,

Table 4
Thermal properties of PLA films containing 1% AP and 2% AT.

	T_g (°C)	T_c (°C)	T_m (°C)	% Cryst.
PLA-AT 2%	58.2 ± 0.6 ^a	74.1 ± 0.1 ^a	142.6 ± 1.5 ^a	13.6 ± 0.5 ^a
PLA-AP 1%	59.2 ± 0.7 ^a	94.6 ± 2.7 ^b	147.4 ± 1.7 ^{ab}	13.4 ± 1.4 ^a
PLA	59.7 ± 0.5 ^a	95.1 ± 2.2 ^b	150.7 ± 1.2 ^b	20.3 ± 0.9 ^b

The means of three replicates plus or minus the standard deviation are shown. Significant difference ($P \leq 0.05$) is represented by different alphabetic letters.

decrease the polymer interaction and cohesiveness, and most likely affect the crystallinity and other physical properties of films, including their flexibility (Laohakunjit and Noomhorm, 2004).

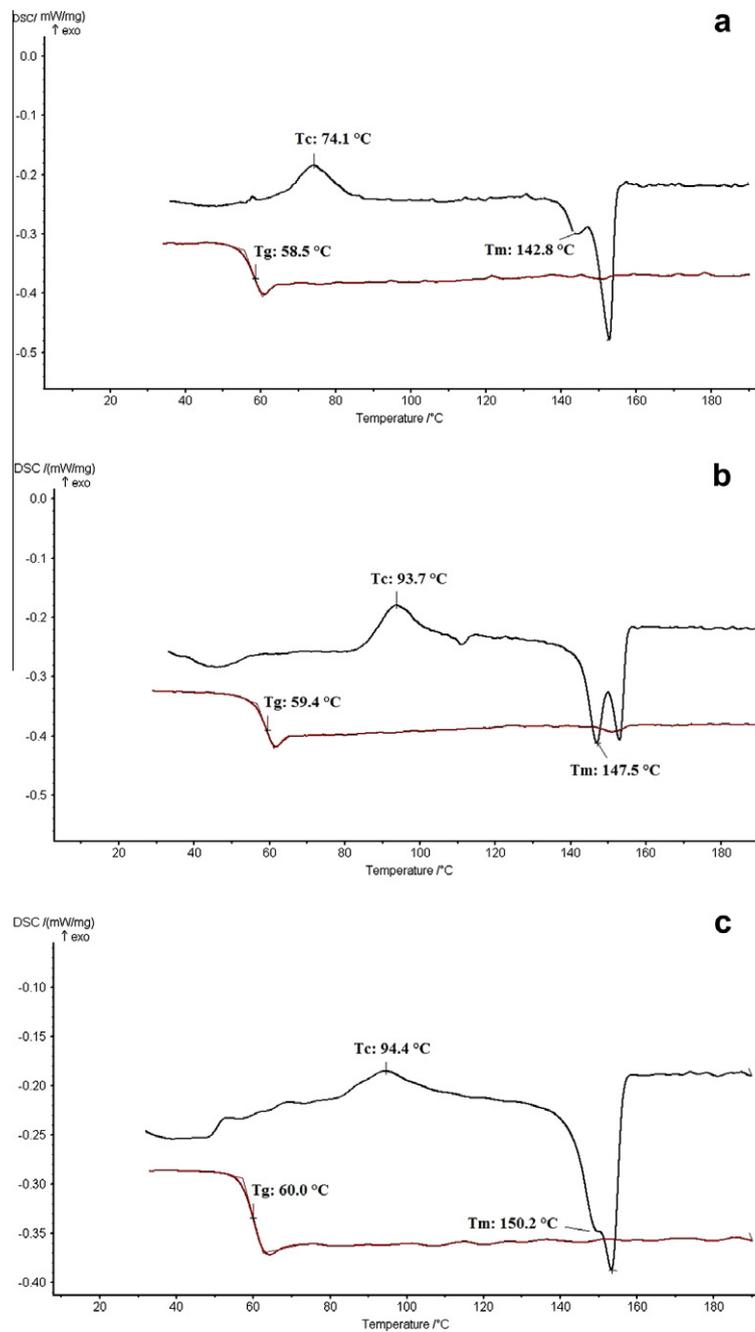


Fig. 5. DSC thermograph of PLA films containing 2% AT (a), 1% of AP (b) and PLA (c). Black and red lines present the first and second heating runs, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5
Mechanical properties of PLA films containing 1% AP and 2% AT.

	Young's modulus (GPa)	Tensile strength (MPa)	%Elongation at break
PLA-AT 2%	2.05 ± 0.09 ^b	53.5 ± 3.6 ^b	4.2 ± 0.2 ^b
PLA-AP 1%	1.82 ± 0.02 ^a	32.7 ± 3.8 ^a	2.5 ± 0.3 ^a
PLA	2.24 ± 0.04 ^b	58.0 ± 2.8 ^b	3.6 ± 0.2 ^b

The means of eight replicates plus or minus the standard deviation are shown. Significant difference ($P \leq 0.05$) is represented by different alphabetic letters.

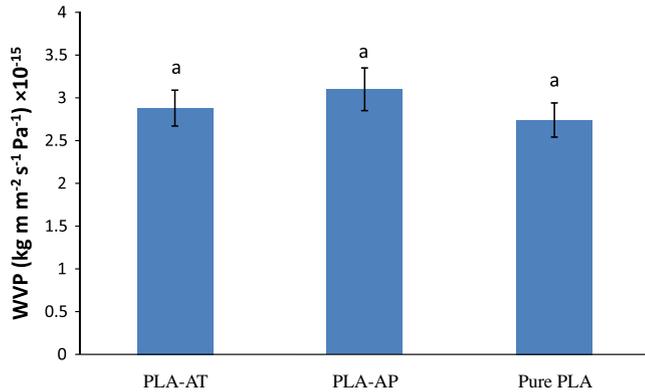


Fig. 6. Water vapor permeability (WVP) of PLA films containing 2% AT and 1% AP.

Two melting peaks can be observed in each thermogram; when two or more melting peaks were observed in a DSC thermogram, we assumed that the lowest melting peak was a real melting peak, while the other peaks were ascribed to the melting of the crystallites formed or thickened during DSC scanning (Tsuiji et al., 2005). The melting point of PLA film was decreased by about 3 and 8 °C by AP and AT respectively. These results are in accord with other research findings demonstrating a shift of T_m to smaller temperatures by the addition of different plasticizers (Pillin et al., 2006; Xiao et al., 2009). The additional melting peaks on thermograms of PLA films containing AT and AP are more noticeable than in pure PLA; this behavior has already been reported for plasticized PLA with poly propylene glycol, and was explained as being related to the reorganization of the crystal structure (Piorkowska et al., 2006). So, it can be supposed that AT and AP participated in lamellar rearrangement which takes place during heating to high temperatures of a specimen crystallized at T_c , and a dual melting peak resulting from this process is observed at 152.7 °C and 152.9 °C for PLA-AT and PLA-AP, respectively.

These behaviors can be explained by the fact that a molecule as a plasticizer can locate at the interface between the amorphous and crystalline parts, thus affecting the actual melting point, or co-crystallize with the polymer during the casting of the films, introducing defects and thus changing the melting temperature (Lopez-Rubio and Lagaron, 2011).

3.7. Mechanical properties

Mechanical properties such as Young's modulus, tensile strength and elongation at break are important factors in studying polymeric films. The mechanical properties of PLA films containing AP and AT are summarized in Table 5. Both AT and AP reduced Young's modulus by about 8% and 19%, and tensile strength by about 8% and 43% respectively. AT as a plasticizer increased elongation at break by about 16% related to its lower T_g , but AP had an inverse effect, a reduction of about 30%, which was probably due to its surface crystallization. Increasing the crystallinity of a polymer can lead to improvements in its stiffness, strength, heat

deflection temperature and chemical strength; an amorphous form decomposes quicker in a landfill or compost environment than a crystalline one (Harris and Lee, 2008). It can be concluded that one reason for the negative effect on mechanical properties of adding AP and AT antioxidants is their propensity to decrease PLA crystallinity. However, crystallinity is not a sole influencing factor, and despite increased crystallinity, lower modulus could result. Lower modulus values (about 500 MPa) were found in films enriched with AT compared to pure PLA (about 900 MPa), even though they showed the same or even higher levels of crystallinity (Gonçalves et al., 2011).

Changes in mechanical parameters made by AT as a plasticizer were expected and are consistent with research findings for other plasticizers (Kulinski and Piorkowska, 2005; Ljungberg et al., 2005). However, as shown in surface characterization section, AT and especially AP were not homogeneously distributed throughout the entire polymer structure, which could lead to polymer inconsistency and be another reason for decreased mechanical parameters. Additionally, there are always some differences between solvent-cast and extruded films. It has been proved that PLA films prepared by the thermocompression method have higher modulus and strength but lower toughness than PLA films prepared by the solvent-casting method (Rhim et al., 2006).

Byun et al. (2010) have shown that the incorporation of AT into PLA film containing 10% of PEG 400 does not significantly change its tensile strength. They concluded that the reason for a 30% reduction in tensile strength and a 240% increment in elongation at break for PLA film containing 1% and 10% PEG 400 compared to pure PLA is their plasticizing effect; however the effect of pure AT without PEG 400 was not considered. The addition of β -carotene to PLA also decreased the Young's modulus and tensile strength by 72% and 54% respectively. However, β -carotene increased the elongation at break to 20 times the level of pure PLA because of its plasticizing effect. The authors proposed that the reason for this drastic increase in elongation could be attributed to the glass transition temperature of polymer at less than room temperature (Lopez-Rubio and Lagaron, 2011).

3.8. WVP

WVP is one of the most important properties in food packaging, mainly because of the noticeable role of water in deteriorative reactions and microbial growth. Water acts as a solvent or carrier and causes texture degradation, and chemical and enzymatic reactions. Measured WVPs of PLA and PLA containing antioxidants are shown in Fig. 6. AP and AT did not significantly change the PLA's WVP. The gas permeability of a semi-crystalline polymer is usually affected by crystalline phase that is impermeable to gases, so the higher amorphous phase makes the polymer more permeable (Drieskens et al., 2009). However, in this study the reduction in crystallinity did not result in WVP augmentation. It has been shown that the water vapor transition rate (WVTR) of PLA films decreases monotonically as crystallinity increases ($\%X_c$) from 0% to 20%, but levels off when $\%X_c$ exceeds 30% (Tsuiji et al., 2006). It was assumed that this is probably due to the higher resistance of "restricted" amorphous regions to water vapor permeation compared with that of the "free" amorphous regions. It seems that AP and AT on the one hand decrease PLA crystallinity and on the other increase the restricted amorphous regions, and that these two phenomena dispel their total effect on WVP.

4. Conclusion

Addition effects of Ascorbyl palmitate (AP) and α -tocopherol (AT) on the physical, mechanical, structural and morphological

properties of solvent-cast PLA were investigated. AP crystallization on the polymer surface changed transparent PLA to an opaque film, which is not desirable in terms of consumer acceptability. Additionally, a 35% loss of AP during film preparation is high, and obviously this loss will intensify by industrial polymer fabrication implying elevated temperatures and pressures. The decreasing effect of AP on PLA contact angle could be attributed to inhomogeneous structure caused by AP crystals. AT was differently incorporated in PLA surfaces because the verso surface of PLA-AT had a higher surface energy than the recto one; this change was observed in both polar and dispersion components as well. XPS results also proved that AT influenced the chemical composition of PLA film on the verso side by changing the concentration of C-(C,H), O=C, O-H, C-O and O-C=O bonds compared to PLA. AFM showed that PLA-AT thin films had a closely similar roughness to PLA, while by adding the AP to PLA film, the roughness parameters including R_a and R_q were increased. The noticeable influence of AT on PLA's thermal properties was found in PLA crystallization temperature. AP and AT slightly reduced PLA crystallinity, which consequently affected the polymer's mechanical properties. Neither of the antioxidants influenced the WVP of PLA film, despite the crystalline surface structure caused by AP. Further studies are needed to investigate the exact interactions of AP and AT with PLA, and also the mode of their distribution in a polymer matrix.

In view of the negative effect of AP on PLA's transparency, the fact that it has no significant modifying influence on PLA's properties, and its high loss during polymer preparation, it can be concluded that it is not a suitable antioxidant for PLA-active packaging, though it could be used directly in food formulations. While AT also had no noticeable modifying effect on PLA film properties either, it could provide a technically safe means of developing PLA as a delivery material.

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Chapitre 3.3

Etude Multi Echelle (Nano, Micro et Macro) des Films de PLA Chargés en Antioxydants

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III. Résultats et Discussion

Chapitre 3.3: Etude Multi Echelle (Nano, Micro et Macro) des Films de PLA ...

Résumé

Dans le but de suivre les modifications des propriétés des films d'acide poly lactique (PLA) contenant 5% d'antioxydants suivants : α -tocophérol (AT), de palmitate d'ascorbyle (AP), butylhydroxyanisol (BHA), butylhydroxytoluène (BHT), propyl gallate (PG) et tert-butylhydroquinone (TBHQ) ont été caractérisés. L'addition de l' α -tocophérol a diminué la température de cristallisation du PLA pur (T_c) de 102°C à 87°C à cause de son effet plastifiant augmentant ainsi l'élongation à la rupture. Aucune modifications des propriétés mécaniques n'a été observé après l'ajout des antioxydants. La cristallisation d'AP sur la surface de PLA a modifié la transparence des films du PLA, les observations sont confirmés par XPS.

La cristallinité des films de APL-antioxydants mesurée par des différentes méthodes (DSC, FTIR et DRX). Les résultats montrent une variation pour certains films. Tous les antioxydants ont augmenté l'intensité Raman à 1774, 1768, 1751 et 1172 cm^{-1} qui signifie que la hausse des phases cristallines et amorphes. De nouvelles bandes sont apparues pour le PLA-PG dans la région de 1700-1600 cm^{-1} . Les micrographes obtenus par MET montrent la précipitation partielle des antioxydants au cours de l'évaporation du solvant et une distribution non uniforme dans la matrice du PLA. L'AFM a démontré que les films minces de PLA-AT ont une rugosité proche des films minces du PLA tandis que les autres ont changé les paramètres de rugosité, R_a et R_q . Globalement, les analyses multi-échelles montrent à l'exception d'AP, les antioxydants n'ont pas d'effets sur la matrice du PLA à cause de l'absence réelle liaison chimique avec les groupements fonctionnels du PLA, ce qui les rends libre dans la matrice.

Chapter 3.3

A Multi-Scale Study (Nano, Micro and Macro) of PLA-Antioxidants Films

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III. Résultats et Discussion

Chapitre 3.3: Etude Multi Echelle (Nano, Micro et Macro) des Films de PLA ...

Abstract

Poly(lactic acid)(PLA) films containing 5% of α -tocopherol (AT), ascorbyl palmitate (AP), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) were characterized to investigate their modification effects and mode of incorporation into PLA matrix. AT decreased the crystallization temperature from the value of 102°C for pure PLA to 87°C due to its plasticizing effect. None of antioxidants had modification effect on PLA mechanical properties unless a little increase of elongation at break caused by AT. AP crystallization on PLA surface made an opaque PLA and largely modified the PLA surface composition approved by XPS. Crystallinity of PLA-antioxidants film measured by DSC, FTIR and XRD methods were not equal for some films. All antioxidants increased the Raman intensity at 1774, 1768, 1751 and 1172 cm^{-1} meaning the increase in both crystalline and amorphous phases. Some new bands for PLA-PG appeared in the region of 1700-1600 cm^{-1} . TEM images showed the partial precipitation of antioxidants during solvent evaporation and their non uniform distribution in PLA matrix. AFM demonstrated that PLA-AT thin films had a near similar roughness with PLA thin films while other antioxidants changed the roughness parameters, R_a and R_q . Globally, multi scale analyses showed that unless AP, other antioxidants freely incorporated in the amorphous phase of PLA matrix and had no real chemical adhesion with PLA functional groups and no specific modification effects.

Keywords: Poly(lactic acid)(PLA), Antioxidants, Incorporation, AFM, FTIR and Raman spectroscopy, Structural characterization.

1- Introduction

Poly(lactic acid) (PLA, $-\text{[CH}(\text{CH}_3)\text{COO}]_n-$) is a well-known biodegradable and biocompatible semi-crystalline aliphatic polyester with a wide range of medical, textile and packaging applications. It has attracted many attentions in recent years due to its ease of production from renewable resources (mainly starch and sugar) and degradation to nontoxic materials.¹ PLA offers great promise in a wide range of commodity applications in medical, food and agricultural fields due to its biodegradability and good mechanical properties.^{2, 3} Tissue engineering also defined as regenerative medicine, is a highly interdisciplinary field that blends principles and innovations from engineering and life sciences for the purpose of developing biological substitutes to restore, maintain, or improve biofunction such as tissue loss or organ failure.⁴ It involves the in vitro seeding and proliferation of relevant cells in or on a scaffold.

PLA, poly(glycolic acid) (PGA), and poly(lactic acid-co-glycolic acid) (PLGA) are a family of linear aliphatic polyesters, which are most frequently used in tissue engineering.^{5, 6} In comparison to other biopolymers, the production of PLA has numerous advantages including: (a) production of the lactide monomer from lactic acid, which is produced by fermentation of a renewable agricultural source corn; (b) fixation of significant quantities of carbon dioxide via corn (maize) production by the corn plant; (c) significant energy savings; (d) the ability to recycle back to lactic acid by hydrolysis or alcoholysis; (e) the capability of producing hybrid paper-plastic packaging that is compostable; (f) reduction of landfill volumes; (g) improvement of the agricultural economy; and (h) the all-important ability to tailor physical properties through material modifications.⁷

Generally, PLA has a high strength, but is brittle, with a very low impact resistance and flexibility, restricts its application as a general-purpose plastic.⁸ Usually, the mechanical properties and chemical stability of a crystalline polymer strongly depend on the morphology and crystal structure. Therefore, the morphology and crystal structure of PLA fibers, films, and single crystals have been extensively investigated by atomic force microscopy, transmission electron microscopy, X-ray scattering, etc, over the past decade.⁹⁻¹¹

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Antioxidants are widely used as food additives to delay the oxidation or to slow the oxidizing reactions rate by a combination of scavenging free radicals, chelating pro-oxidative metals, quenching singlet oxygen and photosensitizers, and inactivating lipoxygenase.¹² Antioxidants also been incorporated in polymer processing to prevent polymer chain breaking and oxidation induced by high temperature and pressure. In our pervious study no modification effects of 4 synthetic antioxidants (1% w/w) on physicochemical, mechanical and barrier properties of PLA were observed.¹³ The most studied antioxidant impregnated to PLA is α -tocopherol due to its good stability and natural origin; α -tocopherol has been investigated to improve the biostability and biocompatibility of different biomaterials. Reno et al. studied the effect of α -tocopherol added to poly((D,L)lactic acid) for medical applications and they verified that it alters the surface wettability.¹⁴ Gonçalves et al. found that even though α -tocopherol did not improve the mechanical and thermal properties, but it promotes an increasing in the oxygen sorption and the convex shape of the isotherms indicating a strong interaction gas-polymer.¹⁵

The aim of this study was to investigate the addition effects of two natural and four synthetic antioxidants including α -tocopherol (AT), ascorbyl palmitate (AP), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) on PLA properties. The effects of a higher concentration of antioxidants on PLA were studied using macro-, micro- and nano-structural analysis methods including X-ray photoelectron, Raman and FTIR spectroscopy, Transmission electron microscopy (TEM) and Atomic force microscopy (AFM). Other PLA-antioxidant films characteristics such as surface analysis, mechanical and thermal properties were also investigated to perform analyses.

2- Materials and Methods

2-1- Materials

Granular PLA (2002D) was purchased from Natureworks[®] Co, Minnetonka (USA); (+)- α -tocopherol (1000 IUg⁻¹) and ascorbyl palmitate (6-O-Palmitoyl-L-ascorbic acid), BHA, BHT, PG, TBHQ, Phosphorus pentoxide, and Magnesium nitrate from Sigma-Aldrich (France), and Chloroform HPLC grade from VWR international, (France).

2-2- Film preparation methods

For each film containing 5% of antioxidant, a solution of 3.5% w/w of PLA was prepared in chloroform and afterwards 5% w/w of antioxidants based on PLA dry matter were added to solution and mixed for 1 hour. Since AP and PG were not completely soluble in chloroform, some ethanol droplets were added in their solution. Then 20 g of polymer solution was cast in Teflon 90 mm Petri dishes (Welch, USA) and the extra chloroform was evaporated in a dark place in hood during 7 days at 25°C. The dried films were kept in a hermetic container containing P₂O₅ powder before each analysis. The final film thickness was 102 ± 6 μm measured (at least 8 points) by a mechanical micrometer (Messmer, UK) according to ASTM D 374-99.

2-3- Thermal properties

In order to determine the transition temperatures including glass transition temperature (T_g), melting temperature (T_m), enthalpies of fusion (ΔH_f) and crystallization (ΔH_c), thermal analysis were performed using a differential scan calorimeter (DSC Q100, TA Instruments-Waters, New Castle, USA). Specimens weighing 5-10 mg were sealed in an aluminum pan and heated under protective nitrogen (40 mL min⁻¹) at the rate of 10°C min⁻¹ from 5 to 210°C then cooled to 5°C and reheated to 210°C with the same cooling-heating rates.

T_g, T_c and T_m were determined from the obtained thermogram. The degree of crystallinity was evaluated according to equation 2:

$$Eq\ 2. \quad \% \text{ Crystallinity of PLA} = 100 \times [(\Delta H_m - \Delta H_c) / \Delta H_f]$$

Where ΔH_m is the enthalpy of fusion; ΔH_c is the enthalpy of crystallization and ΔH_f is the enthalpy of fusion of a wholly crystalline PLA (93.6 J g⁻¹).¹⁶ All tests were accomplished in triplicate.

2-4- Mechanical properties at macroscopic scale

A Lloyd instruments testing machine (AMETEK, LRX, Leicester, UK) was used to determine mechanical properties including tensile strength, Young's modulus and percent of elongation

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at break according to ASTM D882. Before testing, all samples were equilibrated for 48h at $50 \pm 2\%$ relative humidity (RH) in a container using magnesium nitrate saturated solution at $20 \pm 1^\circ\text{C}$. Equilibrated film specimens were mounted in the film-extending grips of the testing machine and stretched at a rate of 25 mm min^{-1} until breaking. The RH and temperature of the testing environment was held at $52 \pm 2\%$ and $20 \pm 2^\circ\text{C}$, respectively.

2-5- X-ray photoelectron spectroscopy (XPS)

The XPS analyses were carried out with a Kratos Axis Ultra spectrometer (Kratos Analytical, Manchester, UK) using a monochromatic Al K α source. All spectra were recorded at 90° take-off angle and analyzed area was about $700 \times 300 \mu\text{m}^2$. Survey spectra were recorded with 1.0 eV step and 160 eV analyzer pass energy and the high resolution regions with 0.1 eV step and 20 eV pass energy, with an exception for the carbon with 0.05 eV step. In both cases, the hybrid lens mode was employed (magnetic and electrostatic). During the data acquisition the Kratos charge neutralizer system was used on all specimens with the following settings: filament current 2A, charge balances 3.5V, filament bias 1.0V and magnetic lens trim coil 0.34A. The C-(C, H) carbon was set to 284.60 eV and therefore used as an internal energy reference. Spectra were analyzed using the Vision software from Kratos (Vision 2.2.6). A Shirley base line was used for the subtraction of the background from each peak. Quantification was performed using the photoemission cross sections and transmission coefficients given in the Vision package.

2-6- Fourier transform infrared spectroscopy (FTIR)

FTIR scans were obtained with a Tensor 27 mid-FTIR Bruker spectrometer (Bruker, Karlsruhe, Germany) equipped a diamond ATR (Attenuated Total Reflection) module specially design for films and a DTGS detector. Scanning rate was 20 kHz and 256 scans were used for reference and samples between 4000 and 600 cm^{-1} . The nominal instrument resolution was 2 cm^{-1} . References were recorded on air. Then, the PLA film was put on the diamond crystal of the optical cell and a pressing was performed for films adsorption onto the crystal. Three to five separate experiments were done for each face of film (face A and face B). All treatments were carried out using OPUS software (Bruker, Karlsruhe, Germany). Raw absorbance spectra were smoothed using a nine-points Savitsky-Golay smoothing function.

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Spectra were cut between 600 and 1900 cm^{-1} . Elastic baseline correction using 200 points was then applied to spectra. Afterwards, the spectra were centered and normalized using OPUS software.

2-7- Raman spectroscopy

The apparatus used was a portable Raman spectrometer (Kaiser Optical Systems, Inc.). The chosen laser had a wavelength of 785 nm which corresponds to the near infra-red domain. The natural phenomenon of fluorescence which usually occurs in case of polymer in the visible range was then reduced. The Raman scattering spectra were performed in a backscattering configuration. The CCD (Charged Coupled Device) allows once the acquisition of a spectrum between 100 cm^{-1} and 3400 cm^{-1} . The spectral resolution was greater than 2 cm^{-1} . The laser power illumination was 400mW, so the acquisition time to obtain a spectrum was short. Spectra were performed in 10 seconds. The acquisition software Labspec[®] adjusted the focus according to the global intensity received on the CCD. Therefore, the maximum of intensity for each sample surface tried to be adjusted. The same software was able to perform the baseline subtraction using always the same polynomial function.

2-8- X-ray Diffraction

Samples were analyzed by wide-angle X-ray scattering (WAXS). The selected tension and the intensity were 30 kV and 40 mA, respectively. The wavelength used is $\text{K}\alpha_1$ copper radiation ($\lambda=0.154$ nm), selected by means of a parabolic multilayer mirror (Osmic) and a cylindrical capillary. In this diffraction system (Instrumentation Electronique, France), the sample axis (radial direction of the film) was perpendicular to the incident X-ray beam. The 2D transmission pattern, intercepted by a Fujifilm image plate normal to the beam, revealed with the adapted scanner (Fujifilm BAS 5000) with a maximum resolution of 25 μm , the image being readily obtained in digital form with a PC microcomputer. Subsequently, the diffraction curve, $I(2\theta)$, analyzed using the PeakFit[®] software (SPSS Inc.) in view of extracting the different components: (i) background, (ii) crystalline peaks and, (iii) amorphous bump (amorphous 'halo'). Following previous authors¹ we fitted the contributions of amorphous

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and crystalline phases by a series of Gaussian curves of different heights and widths. The crystalline rate is obtained using the following equation:

$$\text{Eq. 2} \quad X_c = C/(A+C)$$

Where C and A represent the integrated intensity under signals of the crystalline and amorphous zones, respectively.

2-9- Contact angle and surface energy

The contact angle of the films were obtained at 20°C using a commercial Digidrop meter (GBX Scientific Instruments, France) equipped with a charge-coupled device (CCD) camera 2/3" and a Nikon 60 mm macro lens. This apparatus comprises the software named Windrop⁺⁺ which precisely determines the drop size of the liquid placed on the sample surface. The contact angle was measured by adding randomly 10 drops of ultra pure liquids (2 µl) on the surface of a film disk (80 mm in diameter). Using Windrop⁺⁺ software provided with instrument, measurements of the static contact angle values were fully automated.

The total surface energy of PLA films were determined graphically by the method of Owens-Wendt which is usually used for solids with low surface energy like the polymers. The Owens-Wendt theory divides the surface energy into two components: surface energy due to dispersive interactions and surface energy due to polar interactions. Owens and Wendt proved that the total surface energy of a solid, γ_s , can be expressed as the sum of contributions from dispersion γ_s^d and polar γ_s^p force components ($\gamma_s = \gamma_s^d + \gamma_s^p$). Owens-Wendt equation (Eq. 3) applies the contact angle, θ , data of polar and non-polar liquids with known dispersion γ_L^d and polar γ_L^p parts of their surface energy.^{17, 18}

$$\text{Eq.3} \quad (\gamma_L(1+\cos\theta)) / (2(\gamma_L^d)^{1/2}) = ((\gamma_s^p\gamma_L^p)^{1/2} / (\gamma_L^d)^{1/2}) + (\gamma_s^d)^{1/2}$$

where γ_L is the liquid surface tension.

By plotting $(\gamma_L(1+\cos\theta)) / (2(\gamma_L^d)^{1/2})$ versus $(\gamma_L^p\gamma_L^d)^{1/2}$, the polar and dispersive components of the solid's surface energy were determined by the slope and intercept of the resulting

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graph, respectively. For this measurement, 4 liquids including water, glycerol, ethylene glycol and diiodomethane tested for each PLA film at 23°C and 50% RH.

2-10- Torsional harmonic atomic force microscopy analysis (TH-AFM, HarmoniX™ mode)

The nanoscale mechanical properties of PLA and bio-functionalized PLA with antioxidants films were characterized using torsional harmonic atomic force microscopy (TH-AFM, HarmoniX™ mode). For HarmoniX mapping torsional harmonic cantilevers (HarmoniX Probes, HMX, Bruker corp USA) (resonance frequency 53 kHz, torsional frequency 953 kHz, 1.8 N/m spring constant) were used. HarmoniX measurements were done in air under ambient conditions (28°C) using a Dimension 3100 AFM with a NanoScope V controller and Nano-Scope version 7.30 software (Bruker corp USA). For DMT (Derjaguin-Muller-Toporov) modulus determination the cantilevers were calibrated using a standard PS/LDPE sample.¹⁹ The level of the force applied to the surface was adjusted by the amplitude setpoint, which was used for the feedback control, to 40% of the free amplitude. Imaging was performed at 0.5 Hz scan rates.

The phase, height and DMT modulus images were recorded on several areas of film surface with a scan size of 2 μm^2 and for PLA and PLA-antioxidants films. All offline image flattening and analyses were conducted at the software environment provided by the AFM manufacturer. The statistical parameters related with sample roughness were estimated by the software of the equipment including:

- average roughness (R_a) : the average of absolute value of height deviations from mean surface;
- root mean square roughness (R_q): the root mean square average of height deviations from the mean data plane;
- skewness (S_k): the measure of asymmetry of data or more precisely, the lack of symmetry;
- kurtosis (E_k): the measure of amplitude distribution or more precisely the measure of whether the data are peaked or flat relative to a normal distribution.

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2-11- Transmission electron microscopy (TEM)

The samples were prepared for TEM by putting the PLA films (around 1cm²) in ethanol 99.9 % during 1 h and consecutively in solutions of ethanol-resin MBED 812 with ratios of 2/3-1/3, 1/2-1/2, and 1/3-2/3 during 12 h for each one and finally in pure resin for 1 h. Resin polymerization was done at 56°C during 48 h. Ultra thin film samples (80-100 nm) were cut by an ultramicrotome; Leica Ultracut UC7 (Leica, Nussloch, Germany) and observed by a Philips CM20 transmission electron microscope operating at 200 kV.

2-12- Statistical analysis

The data were analyzed by analysis of variance (ANOVA) using StatGraphics (version 16, StatPoint Technologies, Inc., Warrenton, VA, USA) and differences among mean values were processed by the Student-Newman-Keuls multiple range test. Multiple comparisons were performed through 95% least significant difference intervals ($P \leq 0.05$).

3- Results and discussion

3-1- Thermal behavior

DSC is sensitive to the change in the thermal energy that depends on the meta-stable states of the film structure.^{20, 21} The main application areas of PLA are packaging and biomedical commodities, where, in particular, the glass transition temperature (T_g) and the melting temperature (T_m) largely determine the suitability of the polymer for the application.²² The thermal properties of six different PLA films containing 5% antioxidants and pure PLA are presented in Table 1. The T_g of all samples was located around 59°C, which is agreed by literature data (54-64°C) for PLA.^{23, 24} It can be observed that by adding antioxidants, T_g of PLA-antioxidants films did not vary in compared to PLA. The variation of T_g depends on molecular weight, degree of molecular orientation and crystallinity or the details of T_g measurement.

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The T_m of PLA is around 148°C, addition of antioxidants increased lightly the T_m of PLA. According to Table 2 the crystallization temperature (T_c) of PLA increases by antioxidant addition except for PLA-AT. The reduction in crystallization temperature is consistent with the fact that PLA crystallizes with more ease at lower temperatures due to enhanced chain mobility provided by the AT.²⁵ It can be stated that other antioxidants decreased PLA chain mobility causing higher T_c . Besides the effect of cooling and heating rates, the melting and cold crystallization behavior strongly depend on the molecular chain structure.

All antioxidants differently decreased the PLA films crystallinity. It seems that antioxidants somehow hinder polymer chains lateral rearrangements and hence crystallization in PLA. This similar effect were observed for cellulose fiber and β -carotene in PCL and PHBV, respectively.^{26, 27} The maximum reduction corresponded to BHA. In our previous study 1% addition of BHA, BHT, PG and TBHQ equally decreased the PLA crystallinity from 19.5% to around 14%; this influence can be observed for 5% addition except for TBHQ.

Table 1. Thermal properties of PLA films containing 5% antioxidants.

	T_g (°C)	T_c (°C)	T_m (°C)	% Cryst.
PLA-AT	58.5±0.14 ^a	87.6±0.1 ^c	151.2±0.1 ^a	21.1±0.9 ^b
PLA- AP	59.5±1.13 ^a	105.0±0.1 ^b	151.7±0.1 ^a	19.6±1.4 ^b
PLA-BHA	59.0±0.01 ^a	109.3±3.8 ^b	150.1±1.0 ^a	14.8±0.6 ^c
PLA-BHT	60.0±0.30 ^a	108.7±0.2 ^b	151.3±4.1 ^a	16.3±0.9 ^c
PLA-PG	59.1±0.49 ^a	112.2±0.3 ^b	150.1±0.7 ^a	15.5±1.1 ^c
PLA-TBHQ	59.0±0.14 ^a	108.1±1.2 ^b	149.4±0.8 ^a	21.3±0.8 ^b
PLA	58.0±0.11 ^a	102.6±0.2 ^a	147.9±1.1 ^a	24.2±0.5 ^a

Significant difference ($P \leq 0.05$) is represented by different alphabetic letters.

3-2- Macroscopic mechanical properties

The studied mechanical properties of PLA and PLA-antioxidants films including Young's modulus (GPa), tensile strength (MPa) and elongation at break (%) are presented in Table 2. None of antioxidants had modification effect on PLA regardless a little increase of elongation at break made by AT. One reason for the decrease of Young's modulus, tensile strength by

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addition of antioxidants can be due to reduction in polymer crystallinity. A decrease in the polymer's overall crystallinity can lead to losses in stiffness, strength, heat deflection temperature and chemical resistance.²⁸ PLA-AP had the weakest properties because of surface crystalline structure caused by AP crystals leading to change the PLA homogeneity and reduced mechanical properties. The other reason of mechanical defects of PLA-antioxidants films in compared to PLA could be attributed to non-uniform polymer structure caused by antioxidants. AT acted like a plasticizer and slightly increased the elongation at break; as will be discussed later, the unequal distribution of antioxidants into polymer matrix formed by solvent-casting method, prevented their proper incorporation and featuring their complete influences on polymer properties.

Table 2- Mechanical properties of PLA films containing antioxidants.

	Young's modulus (GPa)	Tensile strength (MPa)	Elongation at break (%)
PLA-AT	1.80±0.08 ^b	37.4±2.8 ^d	8.4±0.8 ^c
PLA-AP	1.42±0.06 ^d	34.6±3.5 ^d	0.5±0.3 ^b
PLA-BHA	1.66±0.03 ^c	44.5±3.1 ^c	3.6±0.2 ^a
PLA-BHT	1.87±0.12 ^b	48.9±3.0 ^{ab}	3.0±0.4 ^a
PLA-PG	1.82±0.04 ^b	43.5±2.7 ^c	4.3±0.7 ^a
PLA-TBHQ	1.78±0.08 ^b	48.8±2.8 ^b	4.1±0.3 ^a
PLA	2.31±0.02 ^a	55.0±2.5 ^a	4.5±0.2 ^a

The means of at least six replicates plus and minus the standard deviation are shown. Significant difference ($P \leq 0.05$) is represented by different alphabetic letters.

3-3- X-ray photoelectron spectroscopy (XPS)

XPS analysis was performed to examine the energy changes and chemical binding states of the polymer surfaces. Fig 1 shows the C1s and O1s XPS spectra of the PLA film. According to Table 3, the atomic composition of the PLA films before and after adding the BHT (C), BHA (D), PG (E) and TBHQ (F) did not significantly change. On contrary, by adding the α -tocopherol (A), and Ascorbyl Palmitate (B) composition of PLA changed in both recto and verso sides for AT and just in the recto side for AP. The major reason for PLA-AP recto side difference is AP crystallization on film surface resulting in an opaque film. The reason for big

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influence of AT on PLA verso film can be attributed to AT precipitation during chloroform evaporation. Antioxidants distribution in PLA matrix will be discussed in TEM section. The C and O peaks were decomposed according to a model for biochemical compounds.²⁹ The C 1s peak was decomposed in three distinct peaks: a peak at 284.60 eV corresponding to C-(C,H) bond, a peak at 286.61 eV due to C-O and O-C=O functions. The O 1s peak was decomposed in two peaks attributed to the O=C, O-H at 531.27 eV and O-C at 532.74 eV. The above results showed that by adding 5% natural antioxidants the hydrophilicity of PLA is significantly changed via increasing carbon composition.

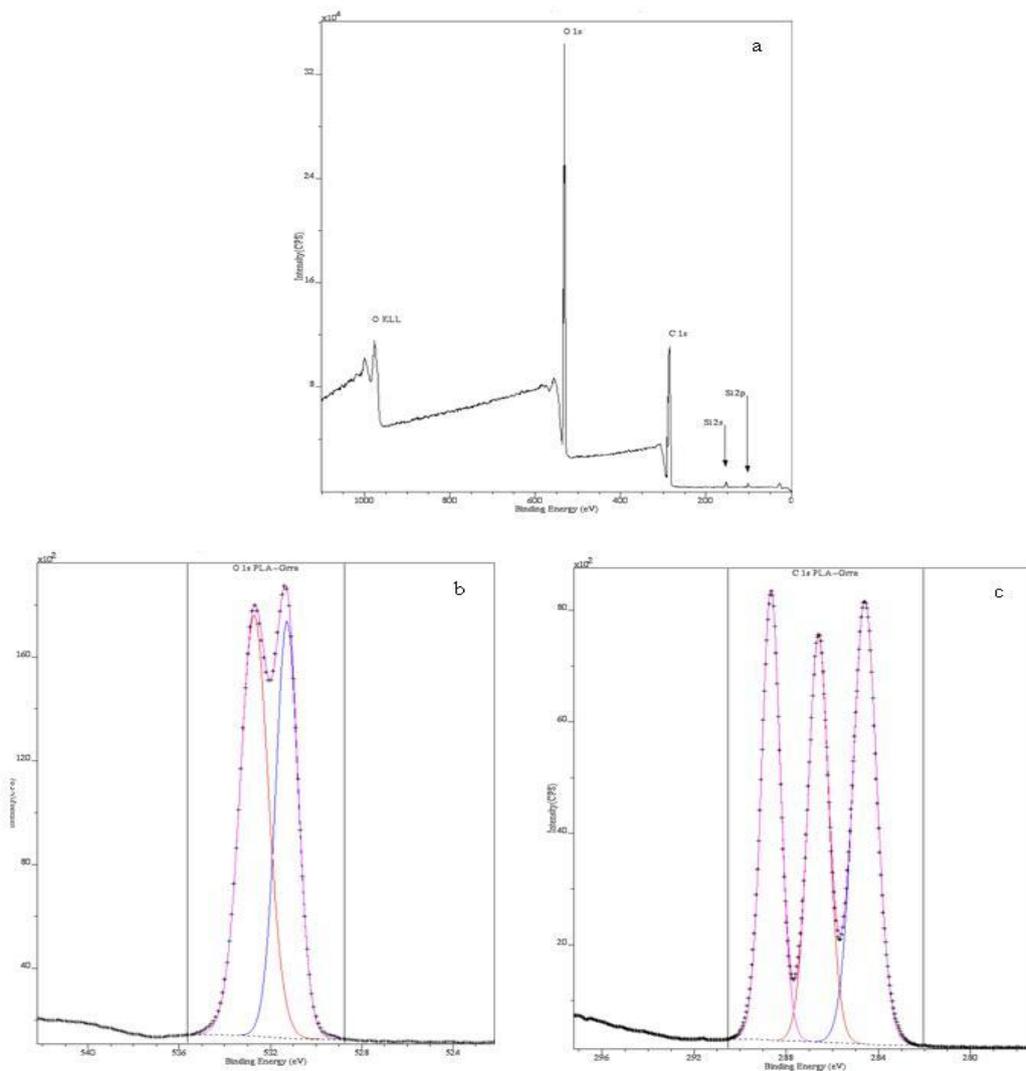


Fig 1- Example of XPS spectra obtained for PLA: (a) survey scan; (b and c) are narrow spectra of C 1s and O 1s levels, respectively.

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After adding the AT and AP, the atomic ratio of O/C decreases from 0.59 to 0.33 for AT (recto: the side in contact with air) and to 0.40 for AP (recto) and from 0.60 to 0.09 for AT (verso: the side in contact with Teflon). By decreasing the O/C ratio, the hydrophilicity of PLA decreases. Table 3 clearly shows that the concentration of the C-(C,H) bond increases after AT and AP incorporation, while the concentration of the C-O and O-C=O bonds decreases. As a result, one can conclude these natural antioxidants mainly influence the C-(C,H) bond.

Table 3. Atomic compositions and concentration of the different chemical bonds of PLA films containing 5% SPAs and natural antioxidants.

	O 1s	C 1s	O/C	C1s C-(C,H)	C1s C-O	C1s O-C=O	O1s O=C O-H	O1s O-C
PLA, R	36.8	62.2	0.59	38.6	30.6	30.8	44.3	55.7
PLA, V	37.7	62.3	0.60	35.4	32.6	32.0	43.5	56.5
A5% , R	25.1	74.9	0.33	61.3	21.7	17.0	46.1	53.9
A5%, V	8.7	91.3	0.09	90.9	6.9	2.2	55.2	44.8
B5% , R	28.5	71.1	0.40	56.0	26.8	17.2	40.4	59.6
B5% , V	36.8	63.2	0.58	36.8	31.7	31.4	41.2	58.8
C5% , R	36.8	62.8	0.58	37.1	31.5	31.4	44.0	56.0
C5% , V	37.3	62.7	0.59	36.2	31.9	31.8	43.5	56.5
D5% , R	36.7	63.3	0.58	35.7	32.3	32.0	42.6	57.4
D5% , V	36.9	63.1	0.58	35.7	32.6	31.7	43.1	57.0
E5% , R	37.2	62.8	0.59	35.5	32.5	32.0	43.4	56.6
E5% , V	36.8	63.2	0.58	35.8	32.4	31.8	42.8	57.2
F5% , R	37.0	62.7	0.59	37.0	31.7	31.2	43.6	56.3
F5% , V	37.1	62.9	0.59	35.9	32.4	31.7	43.0	56.9

A= PLA-AT, B=PLA-AP, C= PLA-BHA, D= PLA-BHT, E= PLA-PG, F= PLA-TBHQ; R= recto side and V= verso side.

3-4- Fourier transform infrared spectroscopy (FTIR)

Fig 2 presents the FTIR spectra of the PLA and PLA-antioxidants samples in the 1900-600 cm^{-1} region.

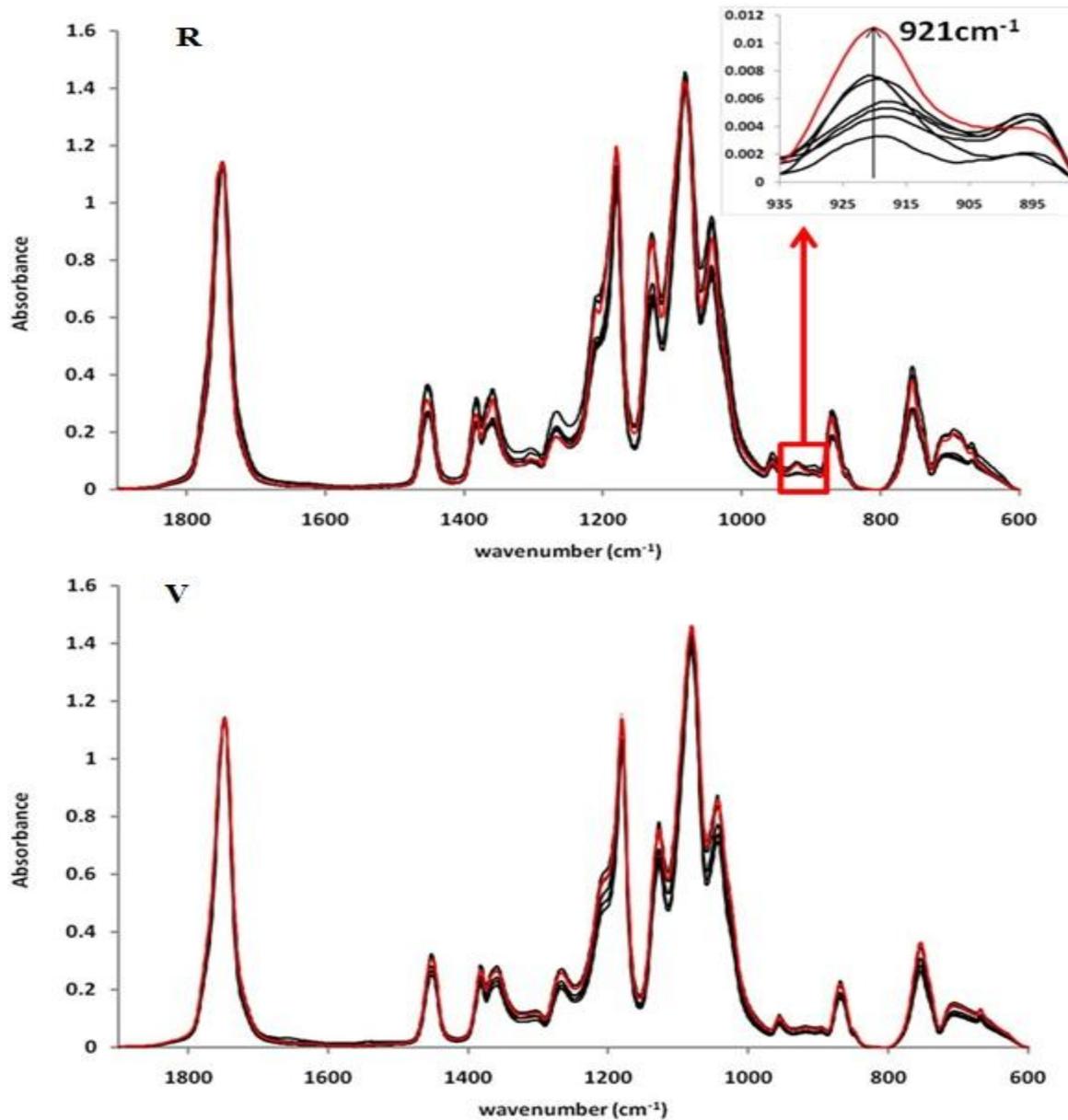


Fig 2- FTIR spectra of PLA films, PLA-antioxidant films are in black and pure PLA is in red (R: recto side and V: verso side).

Table 4 compares the bands assignments collected from the literature and the bands obtained in this study. We can observe the difference between wave numbers assignments in the

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literature and those presented in this study do not exceed more than 4cm^{-1} except for the bands in C=O stretching region that are very broad and represent the molecular conformations.

Table 4- Wavenumbers (cm^{-1}) and vibrational assignments of FTIR spectra of the samples in the $600 - 1900 \text{ cm}^{-1}$ region regions.¹

Wavenumber (cm^{-1})	Assignments ¹	Intensity ²	Ref.
704	$\gamma\text{C=O}$	W	1,32
754	$\delta\text{C=O}$	S	1,32
869	$\nu\text{C-COO}$	S	1,21,32
921 - 955	$r\text{CH}_3 - \nu\text{CC}$	W - W	1,21,32
1044	$\nu(\text{C-CH}_3)$	VS	1,20,30,32
1082	$\nu_{\text{as}}(\text{COC})$	VS	1,20,30,32
1130	$r_{\text{s}}(\text{CH}_3)$	VS	1,20,30,32
1181 - 1210	$r_{\text{as}}(\text{CH}_3) - \nu_{\text{as}}(\text{COC})$	VS - Sh	1,20,30,32
1268	$\nu(\text{CH}) \nu(\text{COC})$	S	1,20,30,32
1302	$\delta_2(\text{CH})$	W	1,20,30,32
1360	$\delta_1(\text{CH}), \text{CH wagging}$	S	1,30,32
1382	$\delta_{\text{s}}\text{CH}_3$	S	1,30,32
1454	$\delta_{\text{as}}\text{CH}_3$	S	1,30,32
1748	$\nu_{\text{as}}(\text{CH=O})$	VS	1,20,30,32

1- δ : deformation; r: rocking; ν : stretching; as: asymmetric and s: symmetric; 2- W: weak; S: strong; VS: very strong and Sh: shoulder.

The absorption bands at 1207 cm^{-1} (due to alkyl-ketone chain vibration) and 921 cm^{-1} (due to flexural C-H bond vibration) are representative of the crystalline structure of PLA.³⁰ For the amorphous sample, the complete lack of 921 cm^{-1} band was shown. This absorption band has been known as the characteristic of α crystal with the distorted 10_3 helix conformation.³¹⁻³³ In this region of the spectra ($1000-800 \text{ cm}^{-1}$), the most intense band is located at 871cm^{-1} that is assigned to $\nu\text{C-COO}$ stretching and also related to the 10_3 helix conformation.²¹ Since the stretched film involves the helical PLLA chains as supported by the appearance of a weak

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helix band at 921cm^{-1} , the difference in transmittance recorded at 1265, 1210, and 1180cm^{-1} can be related to the conformational change of PLLA. It is well-known that PLLA crystallizes into α crystals with the distorted 10_3 helix conformation from solutions or melt. Kister et al. and, Cohn and Younes reported that an absorption band at 921cm^{-1} is characteristic of α crystals.^{31,32} Subsequently, Lee et al. found that this band shows perpendicular dichroism in oriented α films prepared by tensile drawing of an amorphous film at low temperatures.³³ Recently, Kang et al. assigned this band associated with the transition moment perpendicular to the chain axis to the CH_3 rocking mode combined with a minor contribution from the C-COO and O-CH stretching modes of α crystals.³⁴ We can notice that few analyses and assignments have been made for two crystallization-sensitive bands at 955 and 871cm^{-1} in the region of $970\text{-}850\text{cm}^{-1}$. We can observe that during the crystallization process the intensity of the crystallization band at 921cm^{-1} and 871cm^{-1} increases, whereas the intensity of the amorphous band at 955cm^{-1} decreases.^{20, 21, 30} According to Meaurio et al. the decrease of area of the 955cm^{-1} band is synchronized with the increase of area in 921cm^{-1} band.²⁰ The total crystallinity of the samples can be estimated by using equation (1):

$$\text{Eq. 1} \quad X_{\text{c (FTIR)}}\% = 100 \times [I_{921}/(I_{955}+I_{921})]$$

Where I_{921} and I_{955} indicate the area of the crystalline and amorphous bands, respectively.

The results show by adding antioxidants into PLA, the crystallinity of PLA decreases, in particularly, addition of synthetic antioxidants which are consistent with the results of DSC. We examined the two sides of the films, and we observed no modification of the area of 921cm^{-1} for the side B. FTIR was used to follow the qualitative variation of the degree of crystallinity.³⁵ The quantification of this parameter was carried out by DSC and XRD, as it will be discussed later. These techniques detect different aspects of the crystalline structure that may not be determined with the other alternatives. FTIR is sensitive to the chain conformation or rotational conformers in the crystalline or disordered.

3-5- Raman spectroscopy

The band assignments of Raman spectra of PLA is presented in Table 5. Raman spectroscopy enables one to explore structure and interactions of molecules at the functional group level.

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Table 5- Raman shift and assignments of the Raman bands of PLLA.³⁶

Raman shift (cm ⁻¹)	Assignments
2999	CH ₃ asymmetric stretching
2945	CH ₃ symmetric stretching
2883	CH stretching
1774	C=O stretching (C)
1772	C=O stretching (A)
1768	C=O stretching (C)
1751	C=O stretching (C)
1748	C=O stretching
1454	CH ₃ asymmetric deformation
1388	CH ₃ symmetric deformation
1300	CH deformation (A)
1295	CH deformation (C)
1182	COC asymmetric stretching
1127	CH ₃ asymmetric rocking
1095	COC symmetric stretching
1044	C-CH ₃ stretching
923	CC stretching CH ₃ rocking
873	C-COO stretching
737	C=O deformation (in-plane)
708	C=O deformation (out-of- plane)
410	CCO deformation (C)
396	CCO deformation (C)
301	CCO deformation (C) and C-CH ₃ deformation

A, amorphous; C, crystalline.

It is a source of information to qualitatively and quantitatively elucidate the polymer structure including the extent of conformational and configurational isomerism from band frequency assignments, degree and nature of crystalline/amorphous structure, molecular interactions and conformations of polymers.^{32, 36} Fig 3 shows merged Raman spectra in the range of 3100-160 cm⁻¹ of PLA films (recto and verso) containing antioxidants.

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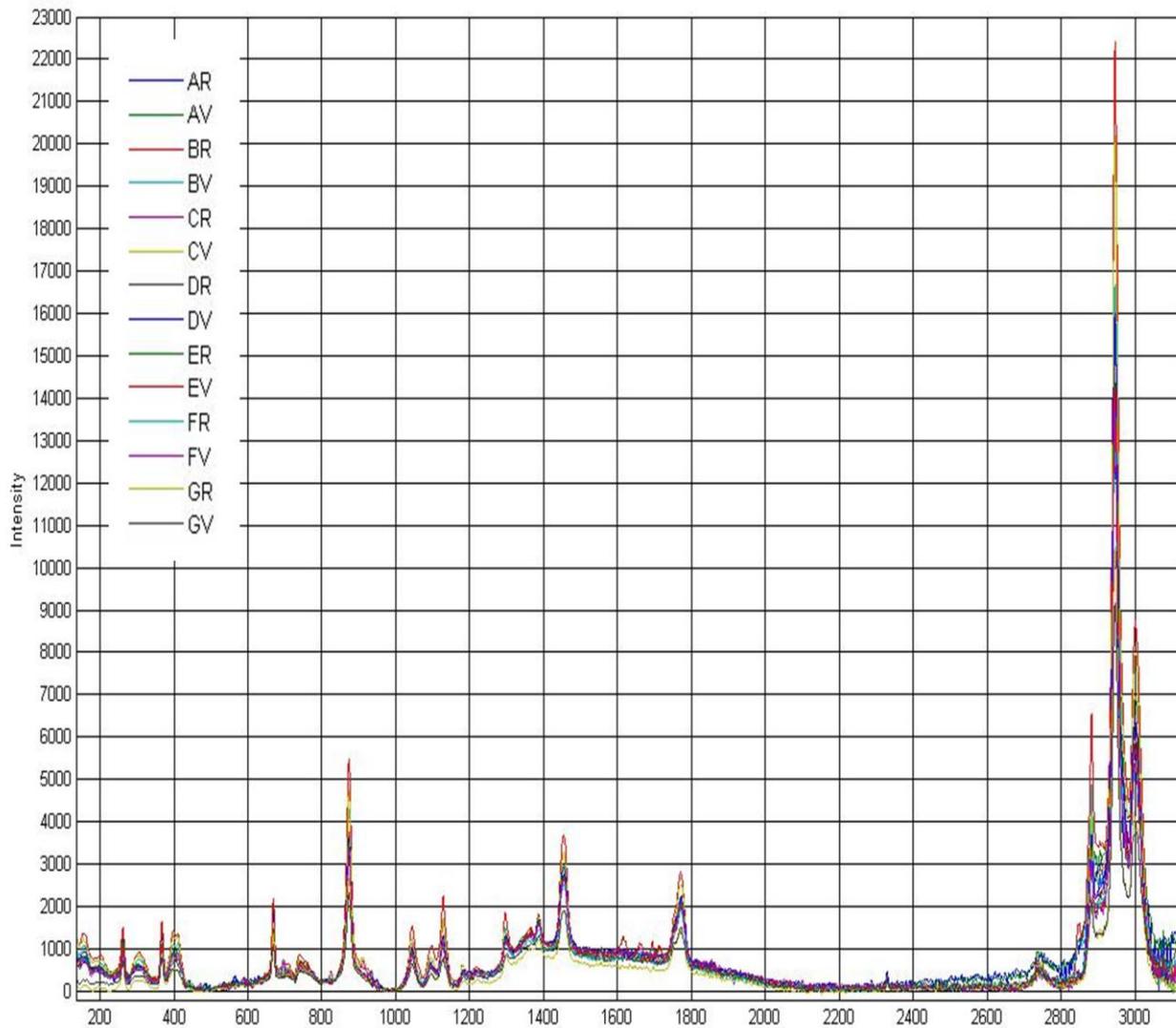


Fig 3- A merged Raman spectra of PLA and PLA-antioxidant film; A= PLA-AT, B=PLA-AP, C= PLA-BHA, D= PLA-BHT, E= PLA-PG, F= PLA-TBHQ; R= recto side and V= verso side, ex AV= PLA-AT verso side.

Approximately all of Raman shifts shown in Table 5 are visible in Fig 3 except the shifts between 1774 and 1748 cm^{-1} having one shift with a shoulder. It is recognized that the crystalline PLLA yields split C=O stretching band near 1774, 1768, and 1751 cm^{-1} and that the amorphous PLLA yields bands at 1772 cm^{-1} . These bands are not distinguishable in Fig 5 but the provided intensity table for each Raman shift shows that all antioxidants increased the Raman intensity at 1774, 1768, 1751 and 1172 cm^{-1} meaning the increase in both crystalline and amorphous phases. Some new bands for PLA-PG (ER and EV) appeared in the region of

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1700-1600 cm^{-1} . Calheiros et al. reported that PG had a C=C stretching vibrations from the aromatic ring at 1650-1600 cm^{-1} ,³⁷ so these bands can be attributed to the phenolic ring of PG, some weak bands are also visible can be attributed to other antioxidants. The Raman intensity of PLA in the regions of 300, 400 and 1100 cm^{-1} was noticeably lower than those of antioxidants explaining their acquisition to the antioxidants. In general, for phenols the in-plane bending vibrations lies in the 1150-1250 cm^{-1} region. The strong band observed both in IR and Raman at 1150 cm^{-1} was assigned to OH in-plane bending vibration.³⁸ Chinna Babu et al. showed that the OH out-of-plane deformation vibration for BHT phenol lies in the 290-320 cm^{-1} region for free OH which is consistent with the antioxidants bands in this region.³⁹ In one hand no specific new band detected in PLA films containing antioxidants and in other hand the Raman intensity of all bands were increased by addition of antioxidants. So one can conclude there is no molecular interaction or binding between PLA and added antioxidants confirming the free state of antioxidants in PLA matrix.

3-6- X-ray Diffraction (XRD)

In this part, the crystalline nature of PLA and PLA-antioxidants was checked by X-ray diffraction. XRD is a method capable of probing long-range order in polymer crystals, and it is therefore used for example to follow changes in the crystallinity of PLA monofilaments.⁴⁰ Methods such as small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) are useful in characterizing the crystalline morphology of PLA, and they have been applied, often in combination with DSC techniques for example in the characterization of biaxially oriented PLA films and PLA stereocomplexes. The PLA diffraction profiles obtained by WAXS shows essentially a broad diffusion band characteristic of the amorphous phase and small crystalline peaks at $2\theta = 16.48, 18.97, 21.29$, in agreement with other studies.^{32, 41, 42}

PLA pattern was characterized by a broad intensity maximum appearing approximately at $2\theta = 16.48^\circ$, indicating an amorphous structure for this sample. Consequently, the degree of crystallinity obtained for PLA with this technique is 44.2%; the discrepancy being due to simplifying assumptions in both methods, namely: (i) simple crystallization/melting balance for DSC and, (ii) perfect crystalline order for WAXS.⁴² Crystallinity is an important factor affecting the glass transition dynamic and enthalpy relaxation behavior through confining the

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segmental mobility of amorphous phase. We compared the % crystallinity obtained by DSC, XRD and FTIR. Calculated PLA crystallinity according to equation 2 was 24.18% and crystallization temperature (T_c) was around 108°C. In many applications, increasing the crystallization amount of PLA is desired because in its amorphous form, the range of application of PLA is severely limited by its low glass transition temperature. Thus, the crystalline form is required to increase the high temperature stability of PLA.⁴³

Table 6- Crystallinity of the samples measured by different techniques.

	DSC	X-ray	FTIR
PLA-AT	21.1	42.0	44.0
PLA-AP	19.6	28.9	23.0
PLA-BHA	14.8	19.8	20.0
PLA-BHT	16.3	25.0	17.0
PLA-PG	15.5	18.1	19.0
PLA-TBHQ	21.3	24.0	26.0
PLA	24.2	44.2	46.0

3-7- Contact angle and surface energy

The surface energy of a polymer influences its adhesive properties, and is important for processes such as printing and heat sealing in packaging field. Also, PLA is known to be biocompatible and widely used in the field of medicine, its low wettability and surface energy affect cell attachment and proliferation and remain an important issue.⁴⁴ Table 7 presents the contact angle, surface energy, polar component and dispersive component of PLA films with and without antioxidants.

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Table 7- Contact angle and surface energy of PLA films containing 1% SPAs.

	Contact angle (°)	Surface energy (mJ/m ²)	Polar component (mJ/m ²)	Dispersive component (mJ/m ²)
PLA-AT (R)	73.2±2.5 ^a	44.7±2.5 ^c	5.6±0.2 ^a	39.1±3.6 ^d
PLA-AT (V)	77.9±2.7 ^a	44.1±1.3 ^c	3.7±0.1 ^d	40.3±2.3 ^c
PLA-AP (R)	44.5±2.6 ^b	53.2±3.4 ^b	30.1±0.3 ^b	23.2±0.4 ^b
PLA-AP (V)	69.4±1.5 ^a	40.3±2.3 ^a	9.8±0.4 ^c	30.5±1.6 ^a
PLA-BHA (R)	74.7±2.2 ^a	40.0±2.2 ^a	6.6±0.2 ^a	33.4±2.0 ^a
PLA-BHA (V)	73.5±1.7 ^a	39.2±1.3 ^a	7.4±0.6 ^a	31.8±2.1 ^a
PLA-BHT (R)	74.5±2.3 ^a	39.9±1.4 ^a	6.7±0.5 ^a	33.2±3.1 ^a
PLA-BHT (V)	76.7±1.6 ^a	38.4±1.8 ^a	6.1±0.3 ^a	32.4±2.8 ^a
PLA-PG (R)	77.5±2.7 ^a	38.8±2.2 ^a	5.4±0.2 ^a	33.4±1.9 ^a
PLA-PG (V)	75.5±1.7 ^a	38.9±1.1 ^a	6.5±0.4 ^a	32.5±3.1 ^a
PLA-TBHQ (R)	70.5±2.1 ^a	40.4±1.4 ^a	8.8±0.7 ^a	31.7±2.2 ^a
PLA-TBHQ (V)	76.5±2.4 ^a	39.3±2.3 ^a	5.8±0.1 ^a	33.5±1.3 ^a
PLA (R)	75.3±1.6 ^a	36.3±1.1 ^a	6.9±0.4 ^a	29.4±1.6 ^a
PLA (V)	75.1±1.1 ^a	36.4±0.9 ^a	6.9±0.5 ^a	29.6±1.3 ^a

The means of three replicates plus or minus the standard deviation are shown. Significant difference ($P \leq 0.05$) is represented by different alphabetic letters.

As can be observed, the contact angle of the PLA film with antioxidant decreases from approximately 75.3° to the lowest value 44.5° for PLA-AP (recto), 69.4° for PLA-AP (verso), 70.5° for TBHQ (recto) and for other antioxidants, this modification is negligible. This decreasing can be made because of structure and distribution of these antioxidants in PLA.

According to XPS results, the O/C atomic ratio varied for AT and AP. This ratio does not significantly change for the other antioxidants. We cannot compare the XPS results with contact angle values because the XPS method determined the composition of PLA and PLA-antioxidants films at 5 nm whilst the contact angle presents the hydrophilicity/hydrophobicity at the surface extreme. The decrease in PLA contact angle by adding the Ascorbyl palmitate is

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in contract with the hydrophobic nature of AP; this reduction can be attributed to the non-uniform surface structure on PLA surface caused by AP crystals. The PLA hydrophobicity is desired for printing practices and plasma treatments is a usual way for modifying this property.⁴⁵ As well, by increasing the wettability, the cell attachment increases.

The surface energy of a polymer will influence its adhesive properties, and it is also important for processes such as printing and heat sealing for packaging and cell proliferation in tissue engineering. According to Table 7, the surface energy of PLA samples increases due to the addition of the synthetic and natural antioxidants especially for natural antioxidants. The surface energy composed to polar and dispersive components.

3-8- TEM

The TEM images of PLA and PLA-antioxidants films are depicted in Fig 4. It was observed that the antioxidants were not uniformly distributed in PLA matrix and their distribution densities were different it two sides of the film. One hypothesis concerning this non homogeneity which can be stated is the antioxidants precipitation during solvent evaporation. As solvent evaporation occurs in one direction, from down to top surface of the Petri dish, the concentration of antioxidant in remained polymer solution in inner layers increases and simultaneously the first layers of PLA matrix are formed on the most superficial layers into contact with air; so, the antioxidants tend to move into the layers containing the solvent. In the other hand, as solvent evaporation causes the more viscous solution, at the final steps of evaporation the movement of antioxidants is difficult and they are fixed at their places in inner layer of PLA matrix or near the verso side; so, these effects make a non uniform distribution in PLA matrix (Fig 4).

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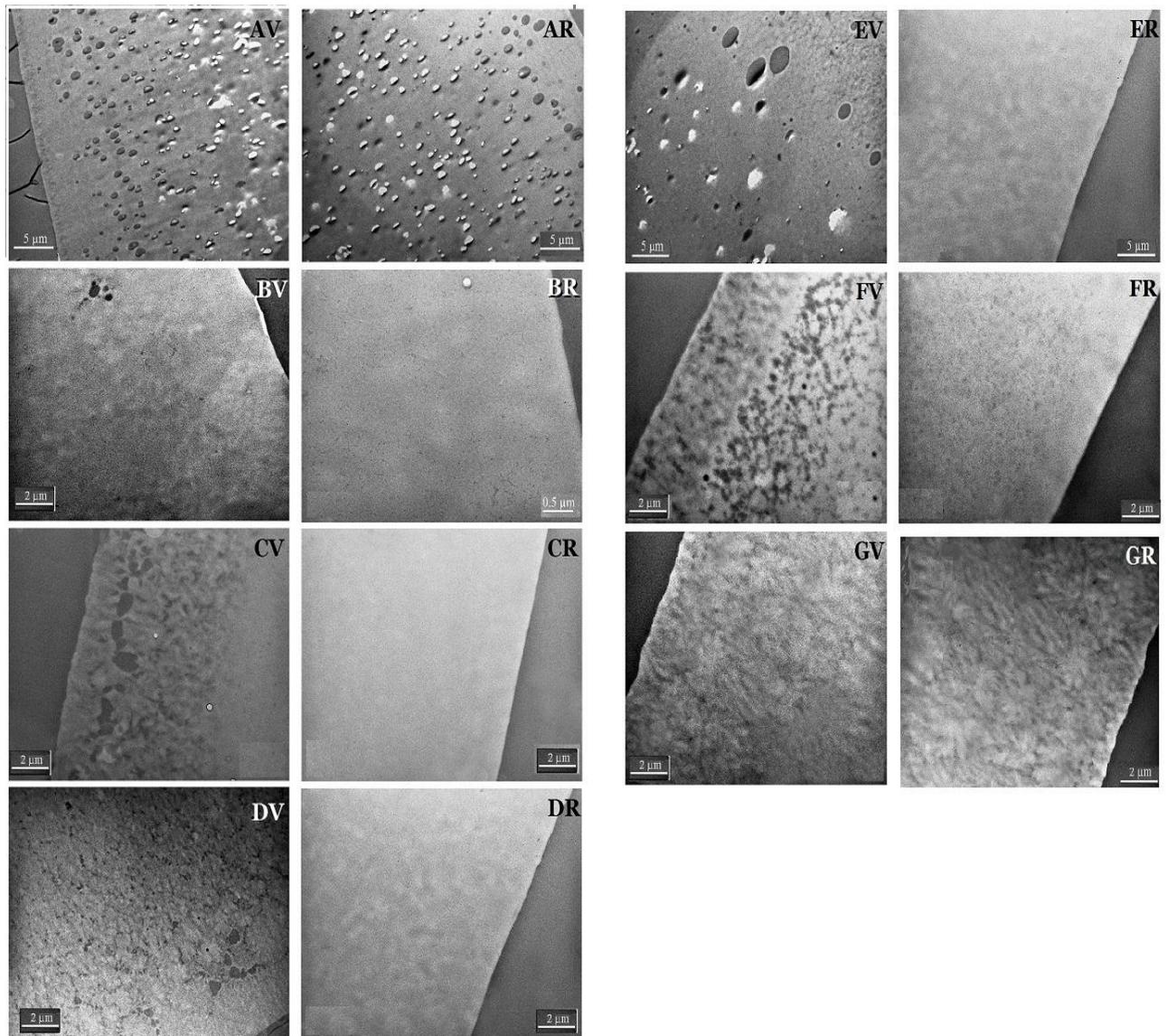


Fig 4- TEM images of PLA films containing antioxidants; AV= PLA-AT verso, AT= PLA-AT recto, BV= PLA-AP verso, BR= PLA-AP recto, CV= PLA-BHA verso, CR= PLA-BHA recto, DV= PLA-BHT verso, DR= PLA-BHT recto, EV= PLA-PG verso, ER= PLA-PG recto, FV= PLA-TBHQ verso, FR= PLA-TBHQ recto, GV= pure PLA verso, GR= pure PLA recto.

As seen in Fig 4 the distribution of AT is more spread than other antioxidants, this is because of its lowest density among other antioxidants leading its fixation at a wider range of polymer layers. Two equal structures of BV and BR confirm that AP did not fully participate in polymer matrix due to its crystallization on PLA surface which changed the transparent PLA to an opaque and rough film. For all other antioxidant two different structures at recto and

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verso sides can be observed confirming the above mentioned hypothesis. One interesting point concerning TEM images is the different structures made by antioxidants incorporation in polymer matrix; BHA, BHT and TBHQ have a sponge like structure in verso side while AT and PG have a porous like structure. These various mode of incorporation can be an influencing factor for interpreting the antioxidants effects on the mechanical, barrier and physicochemical properties of PLA films.

3-9- AFM

The nano-mechanical properties of PLA and PLA-antioxidants films were characterized by force-volume spectroscopy using AFM. Each force curve was triggered to produce the same maximum cantilever displacement; the same force was applied to each sample. The results show that Young's modulus of PLA (2.40 GPa), PLA-PG (2.46 GPa) and PLA-TBHQ (2.47 GPa) are more important than PLA-BHA (2.09 GPa), PLA-BHT (1.94 GPa), PLA-AP (1.1 GPa) and PLA-AT (0.5 GPa). All PLA-antioxidants Young's modulus measured by Lloyd instruments had lower values than those measured by AFM; the largest difference corresponds to PLA-AT. This discrepancy can be attributed to the different mode of measurements, as in stretching method the entirety of film is analyzed while in AFM method surface investigations estimate the Young's modulus. Figure 5 (minuscule letters) shows elasticity profiles of different PLA films using the DMT Modulus imaging mode.

AFM height images of PLA and PLA containing 5% antioxidants thin films are also displayed in Fig 5 (majuscule letters). These images show that the surface microstructures of the films are drastically affected by adding the antioxidants.

PLA thin films (Fig 5G) present a homogenous smooth morphology with $R_a = 1.67$ nm and $R_q = 2.14$ nm. PLA-AT surfaces (Fig 5A) present a pseudo-regular arrangement of nano-dots which can assemble in corolla depleted of nano-dots. These microstructures surfaces are certainly associated with both a difference of mechanical properties and a particular structure of AT. Comparing to with PLA thin films, PLA-AT thin films had a near similar roughness in order of 1.69 nm and 1.34 nm for R_a and R_q , respectively. By adding the AP in PLA film thins, the roughness parameters, R_a and R_q , increase with 5.39 nm and 4.29 nm. A drastically

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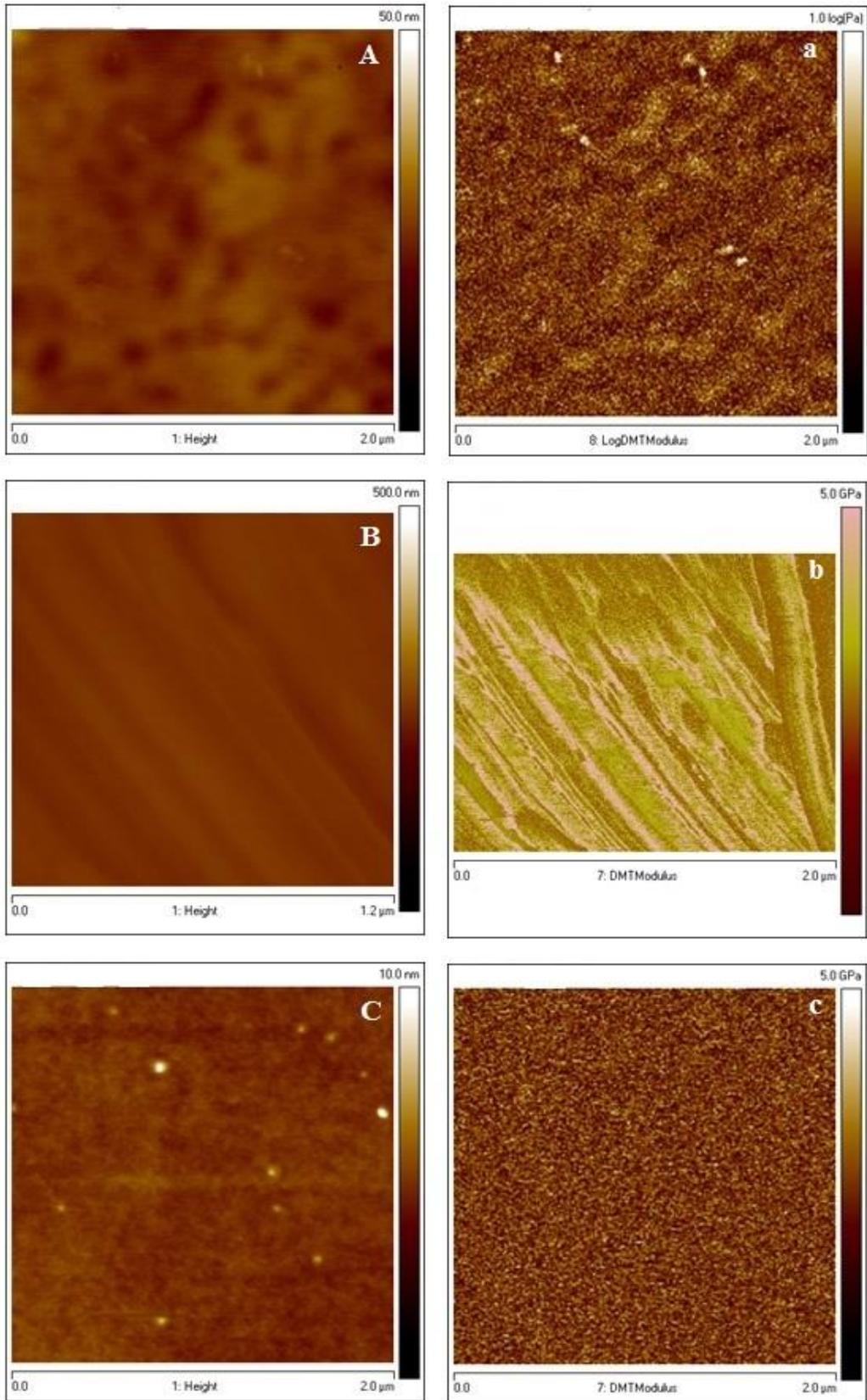
change in microstructure surface compared to AT antioxidant or pure PLA thin films whereas lamellar structure can be observed in Fig 5B.

PLA-BHT and PLA-BHA present also a homogeneous microstructure which can be associated with well-ordering micro fibrils. The surface roughness decrease compared with PLA thin films with $R_a = 0.58$ nm and $R_q = 0.44$ nm for BHT and $R_a = 0.28$ nm and $R_q = 0.19$ nm for PLA-BHA. Adding 5% of TBHQ induced fragmented micro fibrils surface microstructure. Grain-like particles appeared with some remained rodlet shaped. Compared with PLA thin films, PLA-TBHQ thin films had a higher roughness in compared to PLA with 3.95 nm and 2.64 nm for R_a and R_q , respectively. It can be observed a drastically change in microstructure surface compared to PLA films containing BHT, BHA and TBHQ antioxidants. By adding 5% of PG, the surface microstructure varied significantly compared to other surfaces where ellipsoidal structures with a flat center with a thickness in the order of 20 nm and axes $a \approx 1.3\mu\text{m}$ and $b \approx 2\mu\text{m}$ can be seen. It can be supposed that this organization could be attributed to atomic structure of PG antioxidants. As a matter of fact, the roughness of PLA-PG increased in comparing to PLA ($R_a = 1.67$ nm and $R_q = 2.14$).

Recently, tapping mode AFM has been used to investigate the surface structure and interactive force of nano-structured films⁴⁶ and for instance to visualize crystalline morphology of poly(L-lactic acid) thin films.⁴⁷ Therefore, in this study Intermittent Contact AFM (IC-AFM) was performed to get the better topographic images.

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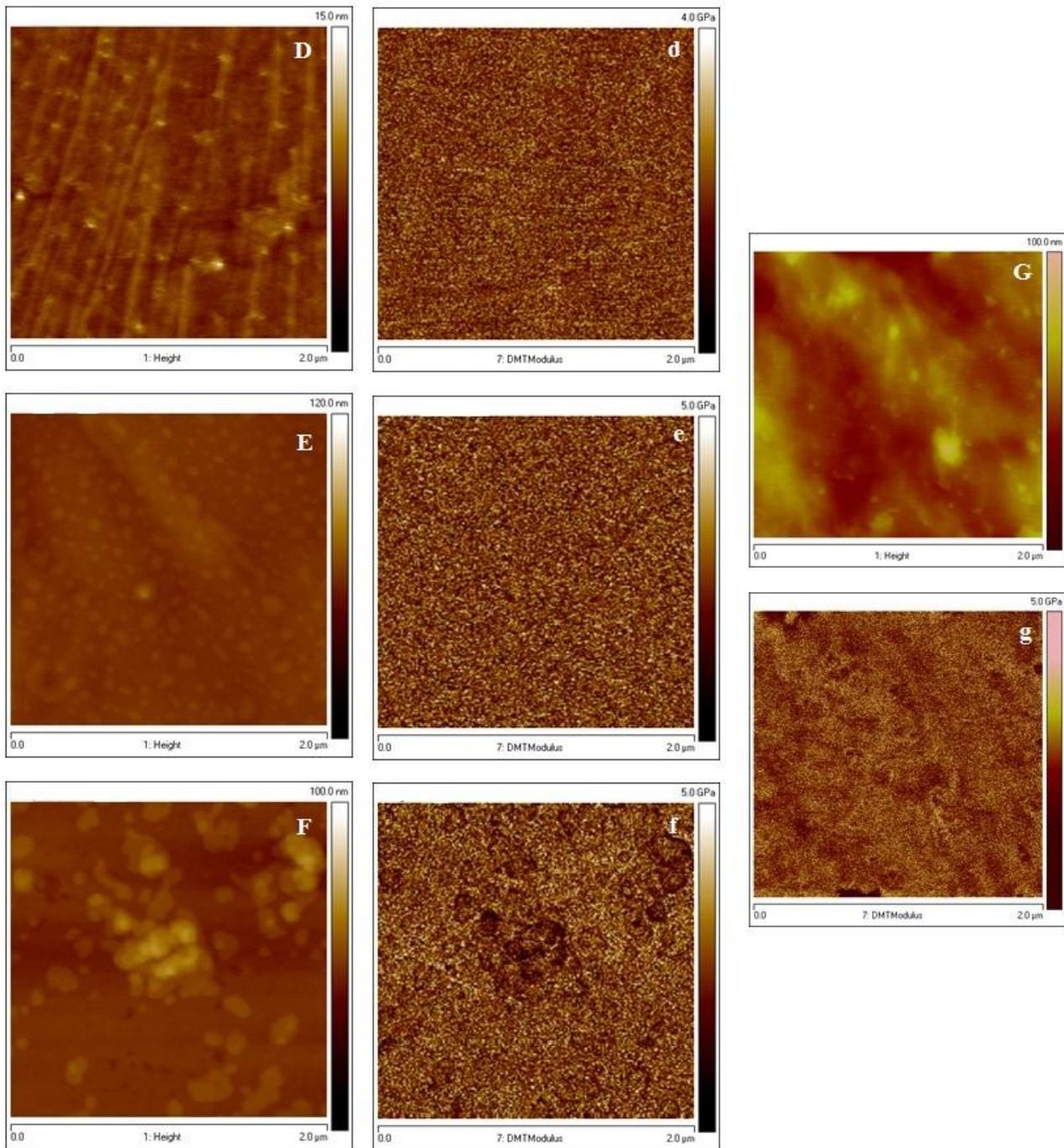


Figure 6- AFM images of PLA thin films, majuscule letters= AFM images of PLA films verso sides, minuscule letters= DMT Modulus images. PLA-AT (A, a), PLA-AP (B, b), PLA-BHA (C, c), PLA-BHT (D, d), PLA-PG (E, e), PLA-TBHQ (F, f), and PLA (G, g).

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4- Conclusion

Macro, micro and nano properties of PLA-antioxidant films were investigated. Diverse thickness layers of PLA-antioxidant films from superficial to profound layers were studied using X-ray photoelectron, Raman and FTIR spectroscopy and AFM and TEM microscopy. The crystallization temperature of PLA increases by antioxidant except PLA-AT due to its plasticizing effect. None of antioxidants showed modification effect on PLA unless a little increase of elongation at break made by AT. One reason for the decrease of Young's modulus and tensile strength by addition of antioxidants can be attributed to the reduction of polymer crystallinity. By adding AT and AP the composition of PLA changed in both recto and verso sides for PLA-AT and just in the recto side for PLA-AP. The major reason for PLA-AP recto side difference is AP crystallization on film surface resulting in an opaque film. All antioxidants increased the Raman intensity at 1774, 1768, 1751 and 1172 cm^{-1} meaning the increase in both crystalline and amorphous phases. Some new bands for PLA-PG appeared in the region of 1700-1600 cm^{-1} . In one hand no specific new band were detected in PLA films containing antioxidants and in other hand the Raman intensity of all bands were increased by addition of antioxidants. XPS showed a large modification of PLA surface made by AP crystals. TEM images showed that unless PLA-AP, other PLA-antioxidant film had two different structures at recto and verso sides due to partial precipitation of antioxidants during solvent evaporation. AFM showed that PLA-AT thin films had a near similar roughness with PLA thin films while other antioxidants changed the roughness parameters, R_a and R_q . Globally, from the results of applied multi scale analyses, it can be concluded that unless AP, other antioxidants non uniformly incorporated in amorphous phase of PLA matrix had no real chemical adhesion with PLA functional groups.

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Chapitre 3.4

Relargage d'Antioxydants Phénoliques Naturels et Synthétiques à Partir d'un Film de PLA Obtenu par Casting

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Résumé

Le coefficient de diffusion (D) et le coefficient de partage (K) de l' α -tocophérol et autres antioxydants phénoliques synthétiques (APS) comme le butylhydroxyanisol (BHA), le butylhydroxytoluène (BHT), le gallate de propyle (PG) et le tert-butylhydroquinone (TBHQ) à partir de film d'acide poly lactique (PLA) dans des aliments simulés ont été déterminés. Les films «PLA-antioxydants» ont été mis en contact avec des solutions d'éthanol 95%, 50%, et 10% à 40°C et 20°C. La relargage des antioxydants a été régulièrement mesurée par HPLC pendant 60 jours. Le poids moléculaire et le Log P des antioxydants et la polarité des simulants sont les facteurs influençant le taux de libération des antioxydants.

Le TBHQ se décompose en 2-tert-butyl-1,4-benzoquinone (TBBQ) et d'autres dérivés de quinones dans le simulant à 95% d'éthanol à 40°C. L' α -tocophérol s'est également décomposé dans le simulant à 50% d'éthanol à 40°C. Les coefficients de D et K des antioxydants étudiés pour trois aliments simulés et deux températures différentes peuvent être utilisées pour créer un emballage actif « PLA-antioxydant » et contrôler en permanence les réactions d'oxydation dans diverses denrées alimentaires.

Chapter 3.4

Release of Natural and Synthetic Phenolic Antioxidants from Solvent-Cast PLA Film

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Abstract

The diffusion coefficient (D) and partition coefficient (K) of α -tocopherol and synthetic phenolic antioxidants (SPAs) including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) from poly lactic acid (PLA) film into food simulants were determined. PLA-antioxidant films were placed in contact with 95%, 50%, and 10% ethanol at 40°C and 20°C. Migrated antioxidants regularly measured by HPLC system for 60 days. Antioxidants molecular weight, Log P and simulant polarity were the most influencing factors on antioxidants release rate. TBHQ decomposed to 2-*tert*-butyl-1,4-benzoquinone (TBBQ) and other quinone derivatives in 95% ethanol at 40°C. α -tocopherol also decomposed to some unknown compounds in 50% ethanol at 40°C. The D and K coefficients of studied antioxidants from PLA for three food simulants and two different temperatures can be used to create PLA-antioxidant active packaging to continually control the oxidation reactions in diverse foodstuffs to ensure higher food qualities.

Key words: Poly lactic acid; Natural antioxidants; Synthetic phenolic antioxidants; Release; Diffusion coefficient; Partition coefficient.

1- Introduction

Different chemical mechanisms are responsible for the oxidation of fats and oils during processing, storage and cooking including autoxidation, photosensitized, thermal and enzymatic oxidation (Choe & Min, 2009). The products of oxidation are almost low-molecular-weight off-flavor compounds which destroy essential nutrients, and produce toxic compounds and dimers or polymers of lipids and proteins (Aruoma, 1998). Antioxidants delay the oxidation or slow the oxidizing reactions rate by a combination of scavenging free radicals, chelating pro-oxidative metals, quenching singlet oxygen and photosensitizers, and inactivating lipoxygenase (Choe & Min, 2006). Direct antioxidants addition to food formulation and dipping food in antioxidants solution are the common way for protecting food lipids oxidation. Most of the primary antioxidants are mono- or polyhydroxy phenols with various ring substitutions such as natural ones including carotenoides, flavonoids, phenolic acids, tocopherols and tocotrienols, and synthetic ones including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), tert-butylhydroquinone (TBHQ) (Wanasundara & Shahidi, 2005). However, there are many foodstuffs which can not be protected by these ways, as they are fresh or raw foodstuffs in which the addition of other substances is not permitted (Tovar, Salafranca, Sanchez & Nerin, 2005). Other limitations of antioxidants direct addition to foods include specific limit of activity because of their incorporation in complex reactions in food systems, and lack of selectivity to target the food surface where most oxidative reactions occur (Gemili, Yemencioğlu & Altinkaya, 2010).

Active packaging generates longer shelf-life foods, lower usage of additives and preservatives in food formulations, higher protection of flavors and higher food qualities (Schaich, Obinata & Yam, 2008). Antioxidant control release packaging provides long food stability to lipid oxidation by in most cases continuously gradual release of antioxidants especially in food surface. Recently, many research studies have been focused on creating successful antioxidant-active packaging with control release ability via using various types of antioxidants, polymers, release modifying agents, and polymer blends and co-extrudes. Wessling, Nielsen, Leufvén and Jagerstad (1999) studied the release of α -tocopherol (AT) in

low-density polyethylene (LDPE) and polypropylene (PP) to different foods and food simulants. They showed a high release rate of AT into fat-containing and alcohol-containing foods from LDPE. Since, AT almost remained intact in PP, they suggested using this antioxidant only for protecting PP from oxidative degradation during polymer processing. Wessling, Nielsen and Giacini (2001) investigated the oatmeal shelf life affected by packaging in two BHT and AT impregnated LDPE films. The results showed that BHT release to oatmeal was faster than AT and had some positive effects on product stability. They didn't recommend AT as an active agent for active packaging of this product because of its high retention in polymer matrix. The study for specific and overall release of rosemary extract from PP to four food simulants including water, acetic acid 3%, ethanol 10% and isooctane was accomplished by Tovar et al., (2005). They showed that rosemary extract can act as an active agent for antioxidant active packaging because its specific and overall release values were 20 times lower than the established limits in the worst cases. Siro et al., (2006) studied the influence of β -cyclodextrin on AT release from LDPE to fatty food simulant. They used β -cyclodextrin as a declining factor for AT release for ensuring a longer-lasting antioxidant effect. The results confirmed that complexation by β -cyclodextrin had a significant reducing effect on AT release which increased AT depletion from LDPE, 2500 h more than blank film (1000h). Briefly, other antioxidant release studies include: BHT and AT release from poly(lactide-co-glycolide) to dry milk products and food simulants (Van Aardt, Duncan, Marcy, Long, O'Keefe & Sims, 2007); catechin, quercetin and thymol from EVOH (ethylene vinyl alcohol) to food simulants (Lopez-de-Dicastillo, Hernandez-Munoz, Gavara, Catala & Lagaron, 2006); BHA, BHT and AT from multilayer co-extruded films made of HDPE, EVOH and LDPE to milk powder (Granda-Restrepo, Peralta, Troncoso-Rojas & Soto-Valdez, 2009); carvacrol from HDPE to olive oil and water (Peltzer, Wagner & Jimenez, 2009); L-ascorbic acid and L-tyrosine from cellulose acetate to water (Gemili, Yemenicioglu & Altinkaya, 2010); antioxidants of barley husk from LDPE to frozen salmon flesh (Pereira de Abreu, Losada, Maroto & Cruz, 2010).

Ascorbyl palmitate (AP) is a fat-soluble ester of palmitic acid and ascorbic acid, and could be used in oils or oil foods. AP is a substance that is generally recognized as safe with no specific limitations or restrictions. Ingestion of this antioxidant would pose no health hazards

because metabolic breakdown yields ascorbic acid and palmitic acid, both normal metabolites; furthermore, it has been shown to be able to regenerate other antioxidants, such as tocopherols (Karabulut, 2010).

Poly-lactic acid (PLA) is a biodegradable aliphatic polyester made from 100% renewable resources, such as corn and sugar beets following fermentation of starch and condensation of lactic acid. PLA is the most extensively researched and utilized biodegradable and renewable thermoplastic polyester, with potential to replace conventional petrochemical-based polymer. PLA offers great promise in a wide range of commodity applications and strongly competes with polystyrene for many food packaging applications (Jamshidian, Tehrani, Imran, Jacquot & Desobry, 2010). There are a little research studies concerning antioxidant release from PLA into foods and food simulants. In a recent study the release of propolis from PLA to water and ethanol to create an antimicrobial-antioxidant active packaging was investigated (Mascheroni, Guillard, Nalin, Mora & Piergiovanni, 2010). The propolis rapid release proposed that ethanol has a plasticizing effect on PLA and would provides more open polymer structure and void spaces for propolis movement.

The aim of this study was to characterize the release behavior and to calculate the release parameters (Diffusion and Partition coefficients) of various natural and synthetic antioxidants including α -tocopherol (AT), ascorbyl palmitate (AP), BHA, BHT, PG and TBHQ from PLA film. Antioxidants release was studied into three food simulating liquids (10%, 50% and 95% ethanol in water) and at two temperatures (20°C and 40°C).

2-1-Materials and Methods

2-2-Materials

Poly (lactic acid) (PLA), 2002 D, was purchased in pellet form from Natureworks[®] Co., Minnetonka (USA); (+)- α -tocopherol (1000 IUg⁻¹), ascorbyl palmitate (6-O-Palmitoyl-L-ascorbic acid), BHA, BHT, PG, TBHQ, and phosphorus pentoxide, from Sigma-Aldrich (France), 2-*tert*-butyl-1,4-benzoquinone from Sigma-Aldrich (USA), Chloroform D (99.8% CDCL₃) from Euriso-Top (France), chloroform, acetonitrile, trifluoroacetic acid (TFA), and methanol from VWR international (France). All solvents provided as HPLC grade.

2-3- Film preparation method

For each film containing an antioxidant, a solution of 3.5% w/w of PLA was prepared in chloroform and afterwards 1% w/w of AP, BHA, BHT, PG and TBHQ, and 2% w/w of AT (2% used because of its more sensibility to light and temperature) based on PLA dry matter were added to solution and mixed for 1 hour at ambient temperature for complete solution of antioxidants in polymer matrix. Since AP and PG were not completely soluble in chloroform, some ethanol droplets were added in their solution. Then 20 g of polymer solution was cast in Teflon Petri dishes (Welch, USA) and the extra chloroform was evaporated in a dark place under hood during 24 h at 25°C then films were placed in incubator (Memmert, Germany) at 30°C for 7 days for complete solvent evaporation. The dried films were kept in a hermetic container containing P₂O₅ powder before each analysis. The final film thickness was 100±5 µm measured (at least 8 points) by a mechanical micrometer (Messmer, UK) according to ASTM D 374-99.

2-4- Quantification of antioxidants in PLA films

The amounts of antioxidants in PLA films after drying were measured by nuclear magnetic resonance spectroscopy (NMR). ¹H NMR data shifts as ppm were collected at 600.13 MHz on an Avance III 600 spectrometer (Bruker, Germany) at 298°K. PLA films dissolved in CDCl₃ and antioxidants amount quantified by comparing the peak area of each antioxidant in a sample and the corresponding peak of the standard with a known concentration. Standard precautions regarding quantization in NMR were taken including identical spectrometers settings, repetition time between two consecutive scans long enough in comparison with relaxation times. All tests were accomplished in triplicate.

2-5- Release tests

Pre-weighed PLA films containing antioxidants (6cm×1.5cm×~100µm) were placed in 10 mL-vials (Agilent, USA) and then 10 mL of each corresponding simulant including 95% ethanol, 50% ethanol, and 10% ethanol in distilled water were added to film samples somehow completely immersed in simulants. The physico-chemical properties of applied

simulants are summarized in Table 1. Aluminum caps equipped with Teflon septum were then tightly sealed by special crimper (CR-20, Chromacol, UK). The vials containing film samples were placed in incubator (Memert, Germany) for being at 40°C and in dark thermostat room for 20°C. The vials were shaken manually two times each day to minimize mass-transfer resistance of antioxidants from the film. These release tests were accomplished in triplicate.

2-6-Measurement of released antioxidants in simulants

Migrated antioxidants measured each 2 days at regular intervals by direct injection of each simulant to HPLC column. HPLC measurements were performed by a HPLC system (Shimadzu, Japan) equipped with a quaternary pump (LC-20AD), an auto-injector (SIL-20AC HT), a UV-vis photodiode array detector (SPD-M20A), a column oven (CTO-20AC) and Labsolution data software.

Table 1- Physicochemical properties of ethanolic simulants.

	pH	Polarity*
Ethanol 10%	6.27±0.01 ^a	14.40
Ethanol 50%	6.42±0.02 ^b	10.82
Ethanol 95%	8.143±0.01 ^c	8.94

* Hansen polarity, data obtained from Molecular Modeling Pro ⁵.

Significant difference ($P \leq 0.05$) is represented by different alphabetic letters.

PLA film samples containing AP, BHA, BHT, PG and TBHQ analyzed using isocratic mode of acetonitrile-0.1% TFA (90%) and H₂O-0.1% TFA at pH 3.0 (10%) at a flow rate of 0.5 mL.min⁻¹. For PLA samples containing AT pure methanol used in isocratic mode at a flow

rate of 0.5 mL.min⁻¹ (Perrin & Meyer, 2003). The simulants (5-50 µl) were injected onto a AlltimaTM HP C18, 3µm (150×3 mm i.d.) column (GRACE, Deerfield, IL, USA) protected by a 2.1×7.5 mm Alltech guard C18 pre-column (GRACE, Deerfield, IL, USA) at 25°C. Each antioxidant identified by comparing retention times and photodiode array spectra of the standards and samples in the range of 200-800 nm. A stock standard solution containing 0.075 mgmL⁻¹ of each antioxidant prepared in methanol and stored at 4°C for 24 h. Purity of each peak was checked so as to exclude any contribution from interfering peaks. Detection of BHA, BHT, PG and TBHQ performed at 280 nm, AP at 255 nm and AT at 292 nm. Quantification was then carried out by comparing the peak area of each antioxidant in a sample with the corresponding peak of the standard. Limit of detection and quantification of antioxidants by HPLC system are presented in Table 2. The linearity of the determination method was measured by injecting standard solution of different concentrations (0.1-5 mgmL⁻¹, 9 points). Repeatability and reproducibility of the HPLC analysis were evaluated by analyzing five replicates of standard solutions in three different days. Recovery tests accomplished by measurement of antioxidants prepared in the simulants. Linear regression analysis showed a linear relationship between peak area and concentration of standard samples (0.1-5 mgmL⁻¹, triplicate 9 points). The correlation coefficient (R²) was 1 for AT and BHT, and 0.999 for AP, BHA, PG and TBHQ. The obtained relative standard deviation (RSD) for repeatability and reproducibility were 0.84% and 2.75% (AT), 1.7% and 5.25% (AP), 0.15% and 0.67% (BHA), 0.10% and 0.13% (BHT), 0.30% and 0.42% (PG) and, 1.5% and 3.62 % (TBHQ), respectively. Recovery experiments were accomplished by spiking known antioxidants standard concentrations within three applied simulants. Antioxidants average recoveries and retention times (RT) are presented in Table 2.

At the end of each working day, the whole chromatographic system was rinsed with water/methanol/acetonitrile 50:25:25 (v/v/v) for 1 h.

Table 2- Antioxidants LOD^a, LOQ^b and recoveries in applied simulants.

	RT (min)	LOD (mg)	LOQ (mg)	95% ethanol	50% ethanol	10% ethanol
AT	8.1	3.4×10^{-8}	3.4×10^{-7}	95.8±0.7	94.3±1.1	92.4±1.2
AP	5.5	4.3×10^{-8}	4.8×10^{-7}	97.2±1.8	90.2±1.3	0 ^c
BHA	2.0	3.7×10^{-8}	3.7×10^{-7}	96.1±0.8	94.7±0.9	92.3±1.5
BHT	4.4	8.0×10^{-8}	8.0×10^{-7}	97.7±0.8	93.7±1.0	90.1±1.4
PG	1.5	1.0×10^{-9}	1.5×10^{-8}	98.3±0.9	96.6±1.0	95.4±1.4
TBHQ	1.7	1.1×10^{-9}	1.7×10^{-8}	99.8±1.0	98.5±0.9	89.8±1.2

a= Limit of detection; b= Limit of quantification and c= AP was not soluble in 10% ethanol and floated on solution surface in form of foam droplets, the solution was filtered through a 0.2 µm filter and analyzed by HPLC.

2-7-HPLC-mass spectroscopy (MS) analysis

The HPLC-MS analysis used for detection of neoformed compounds during release test. The HPLC-MS system comprised the binary solvent delivery pump and a linear trap quadrupole mass spectrometer (LTQ-MS, Thermo Scientific, Inc, USA). LTQ equipped with an atmospheric pressure ionization interface operation in ESI positive mode. Data were processed using Xcalibur software (version 2.1). The operational parameters of the mass spectrometer were as shown below. The spray voltage was 5 kV and the temperature of the heated capillary was set at 300°C. The flow rates of sheath gas, auxiliary gas, and sweep gas were set to 40, 10, and 10 (in arbitrary units per min), respectively. Capillary voltage was 28 V, split lens was -54 V, and the front lens was -4.25 V. All parameters were optimized by infusing the TBHQ standard solution (0.4 mgmL^{-1} in ethanol) in mobile phase. The MS was done in a full scan mode from 50 to 500 m/z. The HPLC column was the same used for HPLC analysis. Solvent A and B were acetonitrile-0.1% TFA and H₂O-0.1% TFA at pH 3.0,

respectively. Gradient conditions include 0-25 min, 30% B; 25-30 min, 90% B; and 30-40 min, 90% B at a flow rate of 0.2 mLmin⁻¹.

2-8- Calculation of diffusion and partition coefficients

The apparent diffusion coefficients (D) of antioxidants were determined from the release versus time data, which was fitted to Fick's second law for an infinite slab in contact with an infinite volume of solvent (equation 1, Crank, 1975).

$$Eq. 1 \quad \frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp \left[- \frac{(2n+1)^2 \pi^2}{L^2} D t \right]$$

Where M_t/M_∞ is the concentration of antioxidant diffused at time t, divided by the concentration of antioxidant diffused at equilibrium and L is the thickness of the film.

For short times ($M_t/M_\infty < 2/3$), equation 1 can be simplified to equation 2 and D can be determined with equation 3.

$$Eq. 2 \quad M_t/M_\infty = 4 (D t / 4L^2 \pi)^{1/2}$$

$$Eq. 3 \quad D = (S.L/2)^2 \pi$$

Where, S is the slope of a plot representing M_t/M_∞ against $t^{1/2}$.

The partition coefficient is defined as the ratio of migrant equilibrium concentration in the polymeric material, C_p , to its equilibrium concentration, in the food phase, C_s (Eq. 4) (Tehrany & Desobry, 2004).

Eq. 4
$$K = C_p/C_s$$

The remained amounts of antioxidants in PLA films after equilibration were measured by NMR.

2-9- Statistical analysis

The data were analyzed by analysis of variance (ANOVA) using StatGraphics (version 16, StatPoint Technologies, Inc., Warrenton, VA, USA) and differences among mean values were processed by the Student-Newman-Keuls multiple range test. Multiple comparisons were performed through 95% least significant difference intervals ($P \leq 0.05$).

3- Results and discussion

3-1- Initial antioxidant concentration in PLA

NMR data showed some antioxidants loss during film preparation and especially polymer solution drying. Table 3 summarizes antioxidants properties and their corresponding losses during polymer preparation. AP had the biggest loss because of its high sensitivity to the light and temperature contrary to our assumed idea about AT. The loss of other antioxidants except BHT was negligibly less than 10% showing the high efficiency of polymer solvent casting method for incorporating antioxidants in polymer matrix. BHT had higher loss than other synthetic antioxidants because of its lower flash point causing some antioxidant evaporation during polymer drying. Some higher loss amounts of AT (34%), BHA and BHT (23%) were already reported in poly(lactide-co-glycolide) (PLGA) film due to their evaporation (Van Aardt et al., 2007). The volatility of antioxidants is desired for dried food applications because of their rapid release via evaporation from polymer surface to target food.

3-2-Release levels at 40 °C**3-2-1- Antioxidants release into 95% ethanol**

The 95% ethanol usually used as a fatty simulant for fats, oil and fatty foods due to its resembling hydrophobicity (Commission Directive, 2007/19/EC). Ethanol is fully miscible with water, and at the same time it provides a more hydrophobic environment.

Table 3- Antioxidants properties and their loss in PLA films during polymer preparation.

	Mw (gmol ⁻¹)	Melting point (°C)	Flash point(°C)	Polarity ^{1A}	Log P ^{1B}	Loss (%)
AT	430.72	2.5-3.5	200-220	7.2	12.1	6.5±0.5 ^b
AP	414.53	116-118	178	7.6	3.5	70.5±5.8 ^d
BHA	180.25	48-55	130	4.0	3.4	3.6±0.6 ^a
BHT	220.54	69-72	118	4.1	6.6	13.9±0.1 ^c
PG	212.20	146-150	187	9.2	1.2	6.6±0.3 ^b
TBHQ	166.22	127.5	171	4.8	3.0	3.1±0.4 ^a

1- Data obtained from Molecular Modeling Pro⁵. A= Hansen polarity, B= Log P or lipophilicity is the logarithm of the ratio of un-ionized antioxidant concentrations in octanol and water. Significant difference (P≤0.05) is represented by different alphabetic letters.

Incorporation of various antioxidants especially food-permitted synthetic ones into 95% ethanol as active agents have been less explored and most of studies were focused on release of aid process antioxidants used for polymer oxidation protection during extrusion processing including Ethanox 330, Hostanos SE2, Irgafos 168, Irgafos 1076, and Sanatox R (Dopico-

Garcia, Lopez-Vilarino & Gonzalez-Rodriguez, 2003; Dopico-Garcia, Lopez-Vilarino & Gonzalez-Rodriguez, 2007; Garde, Catala & Gavara, 1998; Lundback, Hedenqvist, Mattozzi & Gedde, 2006).

As all of used antioxidants in this study have hydrophobic characteristics, so their high release levels into 95% ethanol were expected. No release of AP from PLA-AP film was observed into 95% ethanol; so, it was assumed that the remained AP (environ 20%) was rapidly degraded in polymer matrix in contact with the high temperature. AP degradation investigated using a fresh solution with known concentration protected from light and kept at 40°C. Remained AP amounts after 3, 6, and 24 h were 43.5%, 15.8% and 0%, respectively. For testing the effect of light, AP fresh solution exposed to day light for 3, 6 and 24 h at 20°C; remained AP was 85%, 70% and 14% of its initial concentration after exposing to light, respectively. It can be concluded that AP was completely degraded during polymer preparation and also the primary hours of release study. It can be proposed that AP is not a suitable antioxidant for active food packaging because of its high sensitivity to light and temperature. AP antioxidant activity can be achieved by its traditional direct addition to target foods and also should be protected from light.

The release of AT, BHA, BHT, PG and TBHQ into 95% ethanol, as percent ratio of released antioxidant to its initial concentration in PLA (M_t/M_0), is presented in Fig 1. BHA showed the highest release velocity by having 84.5% of its initial concentration into simulant after 4 h. This can be associated with BHA low molecular weight leading a high mobility of the molecule in polymer chains and also its low melting point causing some molecule transfer into simulant via volatilization. Han, Miltz, Harte and Giacini (1987) concluded that BHA release from HDPE was dependent to temperature; they found that almost all of the antioxidant was lost (greater than 95%) within 1 day at 50°C, within 3 days at 40°C, and within 7 days at 30°C. They also concluded that BHA mass transfer from HDPE was due to volatilization rather than diffusion.

A rapid release of BHT and PG into 95% ethanol was also observed; their total initial amounts in PLA films released after 4 days and equilibrated at this concentration. Due to their hydrophobic nature, these antioxidants are soluble in 95% ethanol and obviously had the

enough liberty to move through PLA matrix towards simulant. BHT also rapidly released from extruded LDPE into 95% ethanol; its total release occurred after 1 week at 4°C and after 1 day at 20°C (Wessling, Nielsen, Leufvén & Jägerstad, 1998).

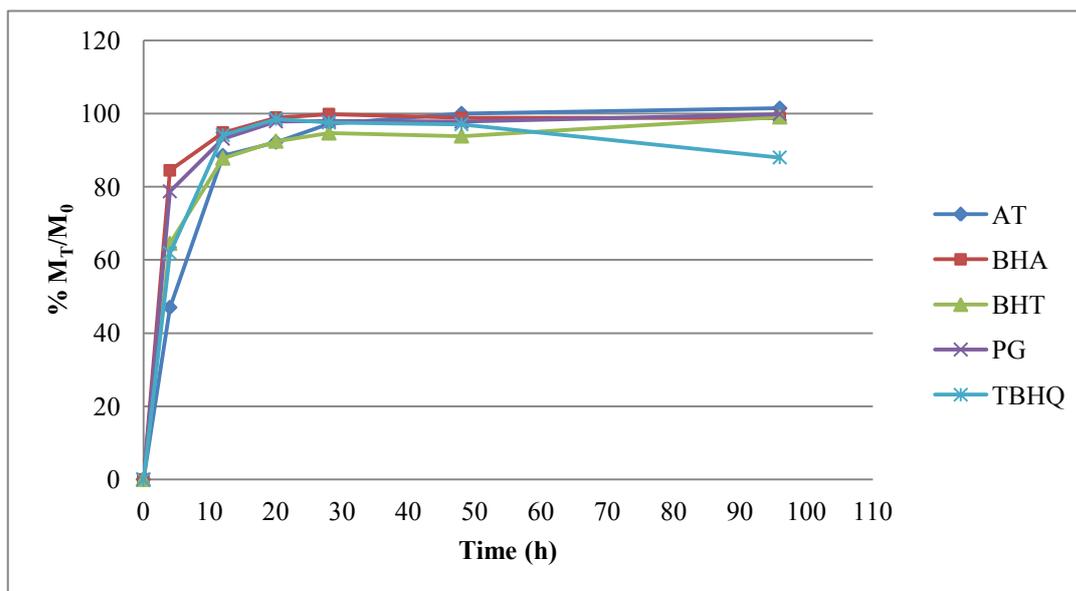


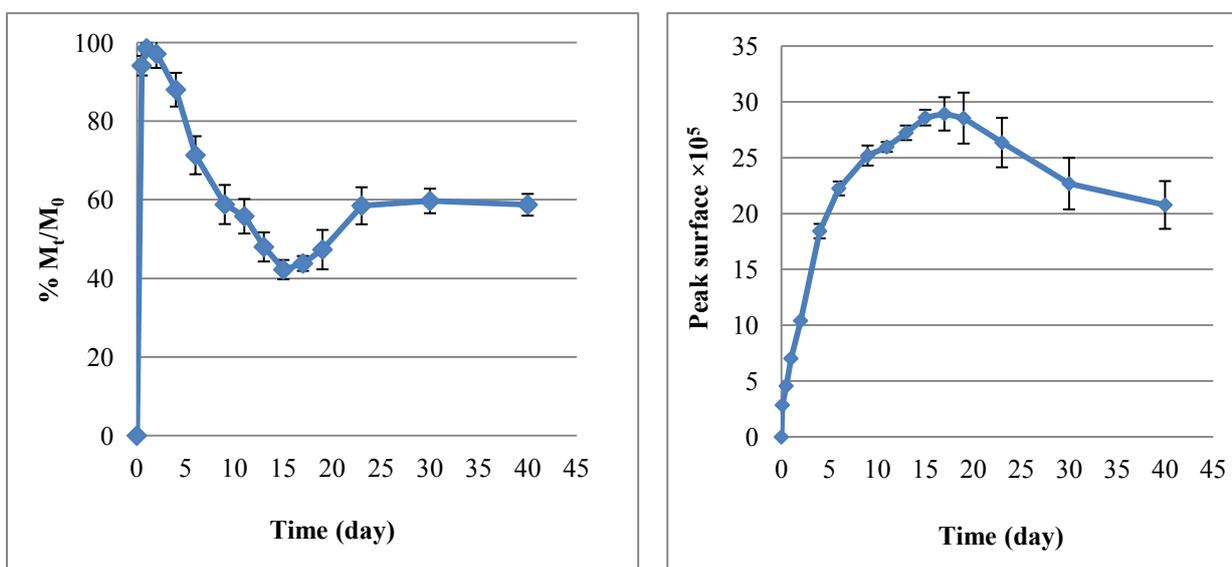
Fig 1- Antioxidants release rate (%M_T/M₀) into 95% ethanol simulant at 40°C.

AT primary release into 95% ethanol was slower than other antioxidants can be because of its higher molecular weight slowing down AT mobility through polymer matrix; however all its amount released after 28 h, demonstrating a high affinity between AT and 95% ethanol. This affinity appears because the molecule of AT has a large hydrophobic alkane chain, with an ether group and a phenol group that can each participate in hydrogen bonding with ethanol. So, the overall nature of AT is hydrophobic resulting higher solubility in ethanol as a hydrophobic simulant. The most important factor for release of migrant from solid polymer phase into simulant liquid phase is migrant solubility in the simulant. Dubbs and Gupta (1998) showed that the solubility of AT in 10% ethanol arises from 0.87 to 698.02×10^6 mol fraction by 71% ethanol concluding by increasing the ethanol fraction in simulant, AT release considerably accelerates. The other factor is the rate of molecule involvement and/or entrapment within polymer matrix; higher molecule involvement in polymer reduces its

release to simulant. Polymer preparation method can directly influence on migrant incorporation into polymer; extrusion process comparing to solvent-casting method provides more structured film and consequently more dispersed migrant. The AT high rate release confirmed no AT involvement in solvent-cast PLA matrix permitting facile AT passage through film into simulant. An entire release of AT into 95% ethanol after 1 week at 20°C from extruded LDPE film and a reduction of 98% after 2 weeks at 4°C confirmed that AT extrusion process had not an entrapping effect on AT (Wessling et al., 1998). However, the authors did not find any AT release from extruded poly propylene (PP) into ethanol 95% presuming higher AT affinity to PP. As PLA has wider polymer structure than LDPE and PP; so, it can be concluded that the polymer nature and its involvement with AT can act as an important factor for AT release, even though a high affinity of simulant. For instance, Heirlings et al., (2004) showed that 82% of AT released into 95% ethanol from both a polar polymer (ethylene vinyl acetate) and a non-polar polymer (LPDE) after 4.6 and 7 days, respectively.

Total amount of TBHQ released into ethanol 95% after 28 h, but its gradual reduction was observed in consequent days describing a probable TBHQ degradation or decomposition in simulant. To investigate all antioxidants degradation/decomposition in 95% ethanol at 40°C, the fresh solution of antioxidants prepared in 95% ethanol and kept at 40°C for 40 days, their concentrations were regularly measured by HPLC. A small reduction for AT (4.4%), BHA (2.2%), BHT (1.4%) and PG (2.9%) was observed after 40 days and no new compound was detected. However, TBHQ reduction was steadily continued until 15th day and started to increase until 23th day and finally stabilized until 40th day. One assumption for this reduction was TBHQ degradation/decomposition in simulant during release test. This assumption approved by tracing all PDA corresponding spectra of the wavelengths from 200 to 800 nm to find neoformed compounds due to TBHQ degradation/decomposition. One peak in isocratic mode was found at retention time of 2.25 min and at 248 nm whose surface consistently increased with decreasing of TBHQ peak surface (Fig 2). The neoformed component appeared from primary days of TBHQ release and increased until 15th day, then remained stable until 23th day, started to decrease until 30th day and finally stabilized until 40th day; as been seen its increasing tendency until 30th day is against the TBHQ reduction.

By using gradient mode (0-60 min 5-95% B, 5 min 95% B) in HPLC analysis, several peaks detected at 248 nm, then their molecular mass measured by LC-mass spectroscopy analysis. New compounds with m/z of 162, 164, 205 and 206 were identified (Fig 3). These compounds were not detected in freshly prepared TBHQ in 95% ethanol. These results globally present TBHQ degradation/decomposition at 40 °C and probable interactions between PLA and TBHQ.



TBHQ detected at 280 nm and 1.66 min

New formed component detected at 248 nm and 2.25 min

Fig 2- TBHQ concentration reduction during release test, while the other compound detected at 248 nm increased. Peak surface corresponds for 2 μ L injection.

III. Résultats et Discussion

Chapitre 3.4: Relargage d'Antioxydants Phénoliques Naturels et Synthétiques ...

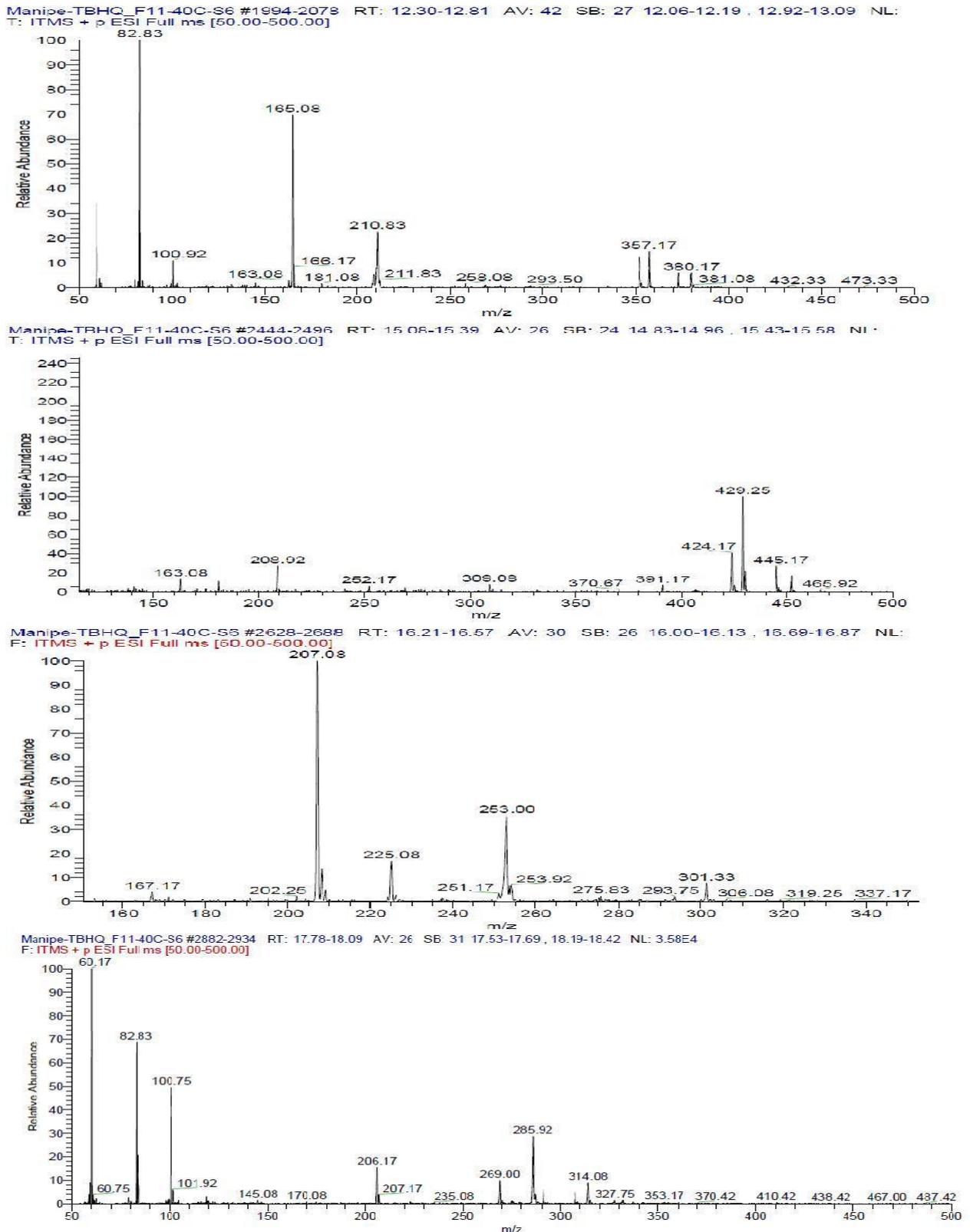


Fig 3- New-formed compounds detected at retention times of 12.30-12.81, 15.11-15.45 and 17.74-18.09 min from PLA-TBHQ film in 95% ethanol at 40°C.

So, we tried to match several TBHQ oxidation products with our unknown compounds. One major product of TBHQ via Cu(II)-induced oxidation is 2-tert-butyl-p-benzoquinone or TBBQ (Mw= 164) previously identified by Li, Seacat, Kuppusamy, Zweier, Yager and Trush (2002). They found that upon mixing 100 μM TBHQ with 10 μM Cu(II), the absorbance of TBHQ at 290 nm decreased with a corresponding increase in absorbance at 252 nm indicating that the reaction of TBHQ with Cu(II) results in its oxidation to TBBQ (Fig 4). The same results were obtained by Bekdeşer, Özyürek, Güçlü and Apak (2011); upon mixing TBHQ with $\text{O}_2^{\cdot -}$, the absorbance of TBHQ at 290 nm was simultaneously decreased by increasing the absorbance of TBBQ at 252 nm concluding oxidation of TBHQ to TBBQ.

Thermal degradation of phenolic antioxidants studied by Hamama and Nawar (1991) showed that the major degradation product from TBHQ oxidation is TBBQ. By passing the pure TBBQ solution through HPLC system, we observed that the UV spectrum of our new formed compound exactly matches with TBBQ spectrum (99% of equality). LC/MS also confirmed that one of neofomed compounds is TBBQ and the others should be other quinone derivatives.

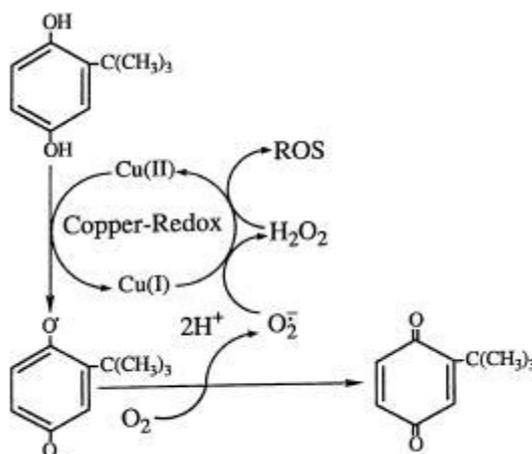


Fig 4- A schematic illustration of the chemical mechanism of Cu-mediated oxidation of TBHQ to TBBQ presented by Li et al., (2002).

These results showed that TBHQ decomposition to TBBQ happens even at low temperatures. Since the TBBQ has not the antioxidant activity, we did not measure its concentration in the simulants. Further studies are needed to fully indentify the other neofomed compounds. It should be mentioned that nothing was detected from pure PLA film in none of the simulants and temperatures

3-3- Antioxidants release into 50% ethanol

The commission of the European unions (EU) recommends using 50% ethanol as a simulant for some milk products because distilled water does not provide an adequate simulant for their fatty character.

The $\%M_t/M_0$ of AT, BHA, BHT, PG and TBHQ are presented in Fig 5. No release of AP was observed into 50% ethanol because of AP degradation at 40°C. The 50% ethanol has a higher polarity than 95% ethanol resulting in slower release of studied hydrophobic antioxidants. The rate of release is strongly limited to antioxidants molecular weight; BHA arrived at its total release after 50 days while AT showed maximum 14% of its total release. By considering the molecular weight, TBHQ could have the highest rate of release but TBHQ showed a different release-oxidation behavior at 50% ethanol. The peak of TBBQ (248 nm) was appeared from the second day and increased until 19th day, then started to decrease until 50th day and finally stabilized but TBHQ concentration was increased at 60% of its initial concentration until 30th day and stabilized at this concentration until 50th day, then started to decrease. It means that TBHQ release and its oxidation to TBBQ simultaneously occurred preventing the exact measurement of released TBHQ in the simulant; So, it can be assumed that TBHQ release rate was higher than the measured concentration. So the order of release without considering TBHQ is BHA>PG>BHT>AT which is in accordance with their inverse order of molecular weights.

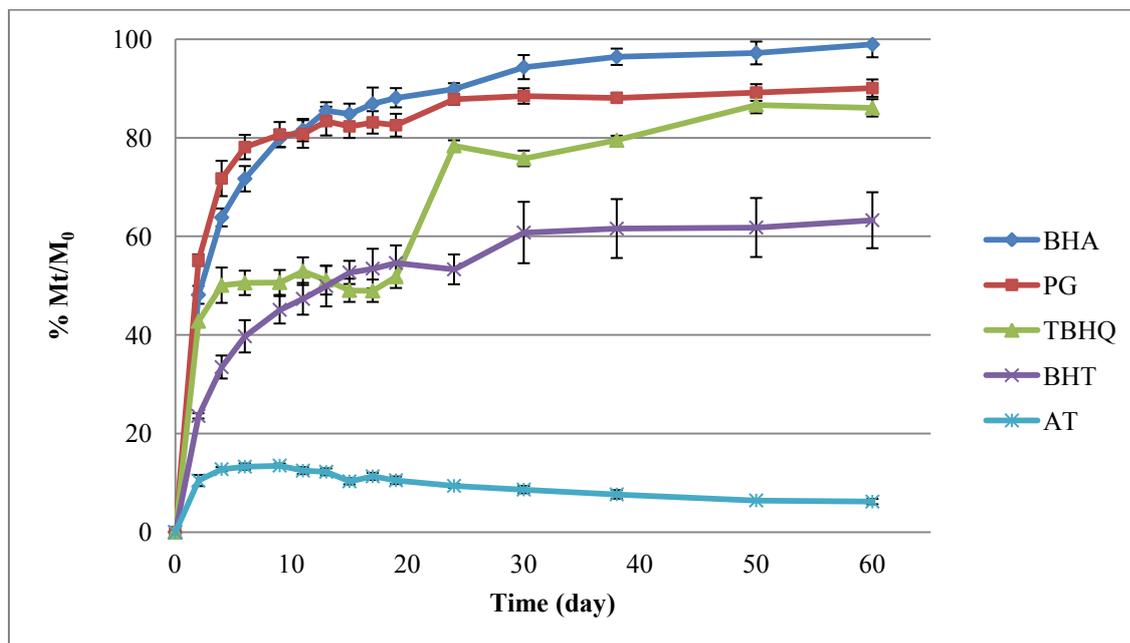
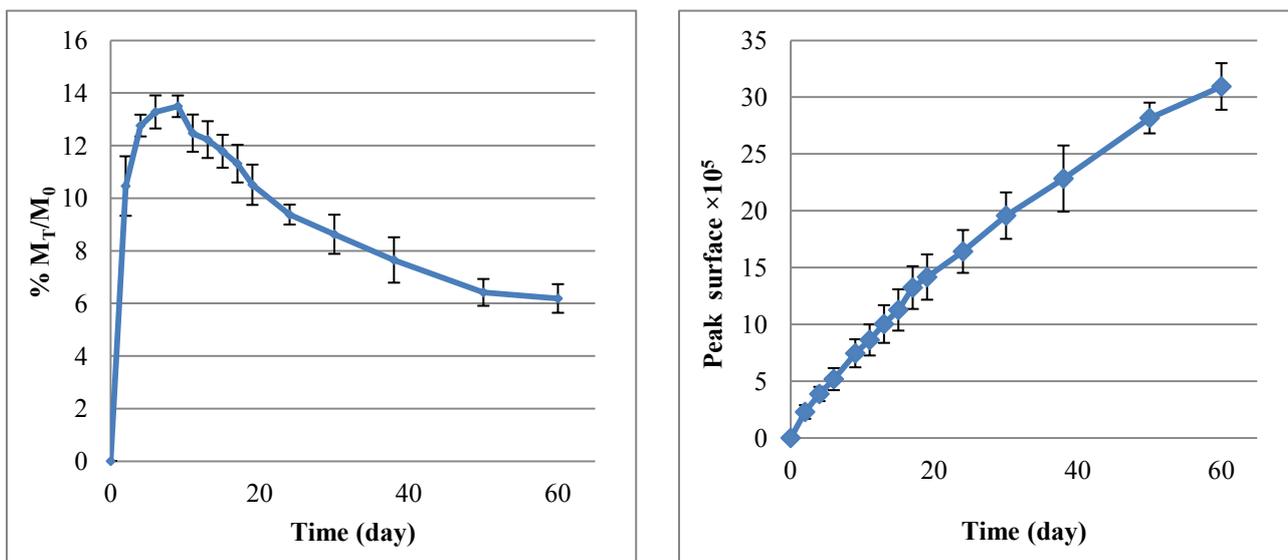


Fig 5- Antioxidants release rate (%M_t/M₀) into 50% ethanol at 40°C.

AT reached to near 14% of its initial concentration into 50% ethanol after 12 days and afterwards begun gradually to decrease. This AT reduction was surveyed by the same method done for TBHQ in 95% ethanol. AT peak tracing demonstrated a large peak at retention time of 5.9 min and 260 nm which had a direct reverse relation with AT reduction in simulant (Fig 6). This new compound appeared from the first days of release meaning simultaneous AT release and degradation/decomposition. LC/MS data using gradient mode showed neformed compounds with m/z of 387, 415 and 423. This is interesting to see that AT degradation happens in 50% ethanol and not in 95% and 10% ethanol. As this new formed compound moves faster than AT in HPLC column, it can be concluded having more hydrophobic properties than AT and further studies are needed to characterize its structure.



AT detected at 292 nm and 8.3 min

Neoformed compound detected at 260 nm and 5.9 min

Fig 6- AT concentration reduction during release test in 50% ethanol at 40°C, while the other compound detected at 260 nm linearly increased.

3-4-Antioxidants release into 10% ethanol

The 10% ethanol is recognized as simulants for alcoholic foods by EU Community and for aqueous, acidic and alcoholic (up to 15%) foods by FDA (CFSAN, 1995). As expected, high polarity of 10% ethanol decreased and for some cases inhibited the release of antioxidants. Among 6 antioxidants, only BHA, PG and TBHQ released into 10% ethanol. The %M_t/M₀ of, BHA, PG and TBHQ are presented in Fig 7.

PG had a faster and higher release rate than BHA showing other influencing factor rather than molecular weight. By considering the Log P of PG (1.2) and BHA (3.4), it can be concluded that lower Log P or lipophilicity permits higher release into simulants with higher polarity; as been seen in Fig 7, the equilibration rate of PG is twice than BHA. High Log P of BHT (6.6) and AT (12.1) prevents their release to 10% ethanol.

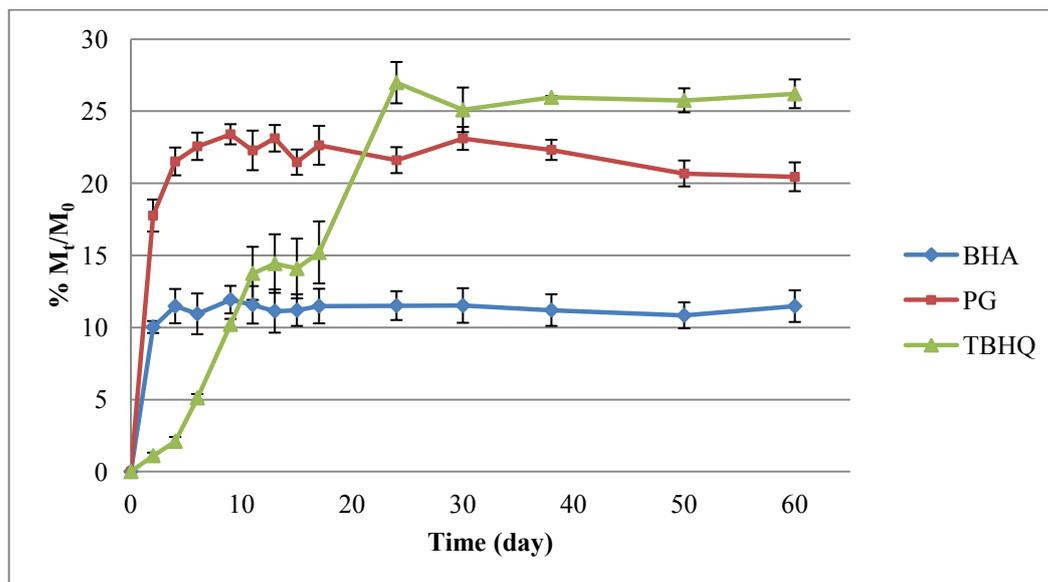


Fig 7- Antioxidants release rate (%M_t/M₀) into 10% ethanol at 40°C.

TBHQ release rate was slower than BHA and PG in first 10 days because of its oxidation to TBBQ, but consequently it had a rapid increase after 17th day. However, a combination of low molecular weight and Log P of TBHQ led its higher release into 10% ethanol than BHA and PG.

3-5-Release levels at 20 °C

3-6-Antioxidants release into 95% ethanol

The temperature reduction obviously had a decreasing influence on antioxidant release; the release rates of all antioxidants significantly decreased. No AP release observed in none of the simulants due to its degradation during release test. The %M_t/M₀ of AT, BHA, BHT, PG and TBHQ in 95% ethanol at 20°C is presented in Fig 8.

The effect of temperature was more remarkable for AT; its equilibration concentration (M_t/M₀) reached to 0.26 after 60 days whilst its total release happened after 2 days at 40°C. The temperature dependence of release is generally described by an Arrhenius type equation:

$$Eq. 5 \quad D = D_0 \cdot e^{-E/RT}$$

Where D is the diffusion coefficient of the antioxidant in the polymer, T is the absolute temperature, R is the general gas constant, E is the activation energy and D_0 is a constant, By reducing the temperature, the antioxidant release obviously declined.

The release of AT from LDPE to 95% ethanol at 6°C and 40°C completely happened after one week confirming no effect of temperature for AT release into 95% ethanol (Wessling, Nielsen & Leufven, 2000). This large difference can be explained by different polymer structures of LDPE and PLA which is not discussed here.

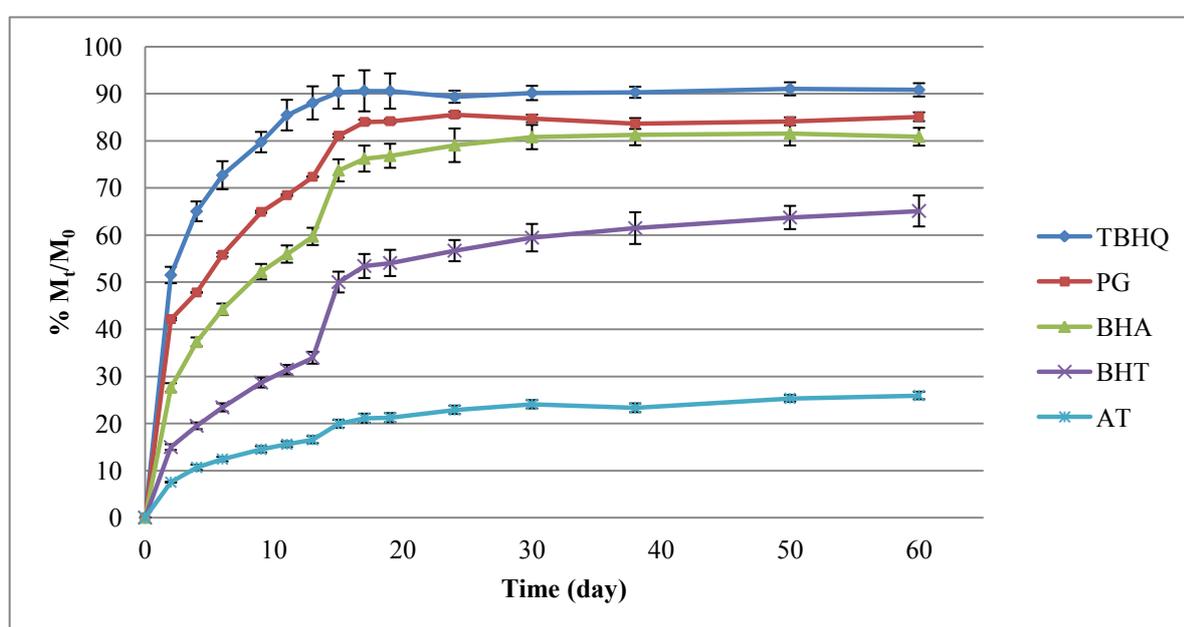


Fig 8- Antioxidants release rate ($\%M_t/M_0$) into 95% ethanol at 20°C.

For other antioxidants the effect of temperature was not as drastic as for AT; the four synthetic antioxidants reached their equilibration concentration at 88% (after 11 d), 84% (after 17 d), 76% (after 17 d) and 59% (after 30 d) for TBHQ, PG, BHA and BHT, respectively. TBHQ was detected in 95% ethanol but its amount gradually increased whilst after 60 days its amount was approximately half of the amount detected at 40°C.

3-7-Antioxydants release into 50% ethanol

As expected, the release rate into 50% ethanol also decreased at 20°C due to reduction in simulant affinity for the antioxidants. The $\%M_t/M_0$ of BHA, PG and TBHQ in 50% ethanol at 20°C is presented in Fig 9. BHA and PG reached their equilibration after 60 days but TBHQ continued its release and TBBQ amount was much lower than the previous conditions. The order of release rate into 50% ethanol at 20°C is in reverse of 40°C describing two different influencing factors including molecular mobility and Log P. Antioxidants mobility at 40°C is higher than 20°C leading higher release of smaller molecules whilst at 20°C both molecular weight and Log P determine antioxidant release.

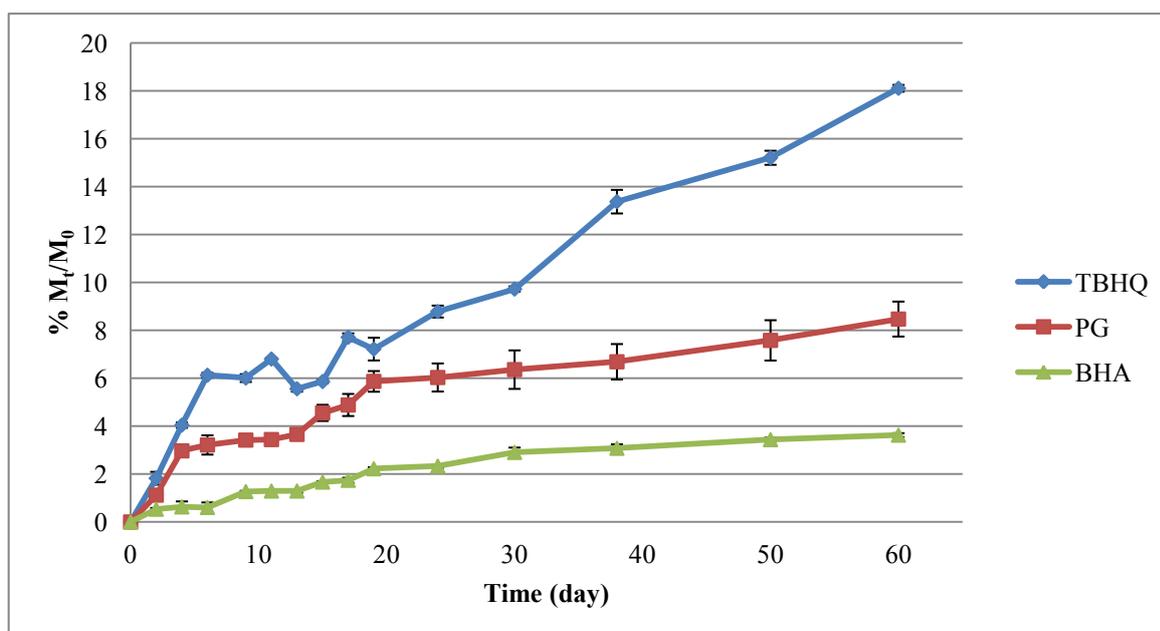


Fig 9- Antioxidants release rate ($\%M_t/M_0$) into 50% ethanol at 20°C.

3-8-Antioxydants releases into 10% ethanol

Among 6 antioxidants, only PG slowly released into 10% ethanol which its $\%M_t/M_0$ is presented in Fig 10. This low release shows that at higher polar condition provided by 10% ethanol, the sole determining factor for antioxidant release is lower Log P. The affinity of

10% ethanol is sufficient just for PG because of its lower lipophilicity than other antioxidants. Because the molecular mobility of antioxidants at 20°C was not provided as well as at 40°C, the release of smaller antioxidants (such as TBHQ and BHA) was not observed. No TBBQ was detected meaning no oxidized TBHQ in simulant and its impossible measurement.

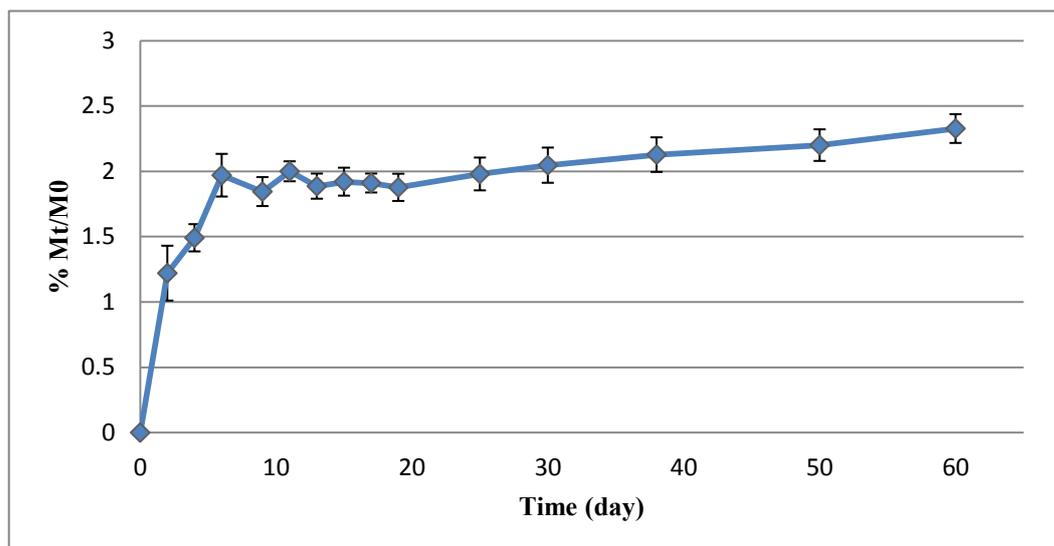


Fig 10- PG release rate (%M_t/M₀) into 10% ethanol at 20°C.

3-9- The D and K coefficients

The D coefficient of studied antioxidants into food simulants are summarized in Table 4. As been seen, the D coefficient decreased by reducing both the percent of ethanol in simulant and the temperature. Otherwise, the effect of temperature on antioxidants release is more influent than the type of simulant. The D coefficient of AT in 50% ethanol at 40°C has the same power as in 95% ethanol at 20°C and also the D coefficient of PG and TBHQ in 10% ethanol at 40°C is higher than in 50% ethanol at 20°C. The effect of temperature can be explained by Arrhenius equation and higher molecular mobility of antioxidants through polymer matrix towards simulant at higher temperatures.

The K coefficient of antioxidants is presented in Table 5. The higher value of the K coefficient appears when the migrant tends more to remains in polymer than to transfer into simulant and vice versa. It is possible to calculate the K coefficient when a thermodynamic

equilibration of antioxidant concentration in both film and simulatant has been achieved. So, for the antioxidants having total or continuous release, K coefficient was not calculated.

Table 4- D coefficients of antioxidants from PLA film into food simulants at 40°C and 20°C.

	<u>40°C</u>			<u>20°C</u>		
	<u>95%</u>	<u>50%</u>	<u>10%</u>	<u>95%</u>	<u>50%</u>	<u>10%</u>
AT	1.49×10^{-9a}	n.d ^A	n.d ^B	2.00×10^{-11a}	n.d ^B	n.d ^B
BHA	4.31×10^{-9c}	1.12×10^{-10a}	2.28×10^{-10a}	3.21×10^{-11b}	7.40×10^{-12a}	2.28×10^{-10b}
BHT	3.12×10^{-9b}	5.84×10^{-11b}	n.d ^B	1.60×10^{-11a}	1.00×10^{-11b}	n.d ^B
PG	3.90×10^{-9b}	1.28×10^{-10a}	2.78×10^{-10a}	6.12×10^{-11c}	7.56×10^{-12a}	8.16×10^{-11a}
TBHQ	n.c	n.c	n.c	n.c	n.d ^A	n.d ^B

A= not determined because of antioxidant decomposition and inability to measure its real value; B= not determined because antioxidants did not reach to its equilibration until 60th day. Significant difference ($P \leq 0.05$) is represented by different alphabetic letters (the standard deviations are not presented).

For example, small value of K coefficient of α -tocopherol in 50% ethanol at 40°C compared to its large value in 95% ethanol at 20°C shows that the majority of α -tocopherol remained in PLA at 20°C which is not in accord with the aim of active packaging.

The D and K coefficients could be used to predict the velocity and the rate of antioxidants release into different foodstuffs having diverse properties as well as the fat, alcohol, and organic acid, and to control the various rates of oxidation at different temperatures. Generally, the total and rapid or zero release of antioxidant from polymer to foods are in contrary with continuous control of oxidation in which gradual release is desired. Further investigations either in polymer matrix or type of antioxidant should be considered for total and rapid or zero release cases .

Table 5- K coefficients of antioxidants in food simulants at 40°C and 20°C.

	<u>40°C</u>			<u>20°C</u>		
	<u>95%</u>	<u>50%</u>	<u>10%</u>	<u>95%</u>	<u>50%</u>	<u>10%</u>
AT	infinite	531±41 ^c	n.d	2293.1±185.3 ^c	n.d	n.d
BHA	infinite	infinite	1133± 96.8 ^b	38.6±4.1 ^b	1880.5±196.1 ^b	n.d
BHT	infinite	53.1±3.2 ^b	n.d	44.2±3.9 ^b	n.d	n.d
PG	infinite	36.3±1.8 ^a	443±26.7 ^a	20.0±1.1 ^a	887.3±51.6 ^a	1646±89.0
TBHQ	infinite	n.c	n.c	n.c	n.d	n.d

n.d= Not determined, because the antioxidants did not reach to their equilibration until 60th day, n.c= not calculated because of TBHQ decomposition. Significant difference ($P \leq 0.05$) is represented by different alphabetic letters.

3-10- Factors influencing the antioxidants release from PLA

According to Limm and Hollifield (1996) several factors influence antioxidants diffusion through polymer including: (1) related-polymer factors such as polymer morphology, crystallinity, molecular weight distribution, density, and orientation which eventually determine sizes and shapes of microcavities and their distribution, (2) related-antioxidants factors such as their molecular size, shape, density, polarity, and solubility, (3) factors related to the interaction of a polymer and antioxidant such a plasticizing or antiplasticizing effect, and (4) factors related to temperature such as T_g and T_m of polymer. They did not consider the potential attracting effects of food or food simulant on additive diffusion.

In this study by using different kinds of antioxidants, simulants and temperatures, all of above mentioned factors can influence the antioxidant release from PLA into simulants. For example two different antioxidants at the same condition such as BHA and TBHQ in 50% ethanol at 40°C, or one antioxidant at two different simulant such as BHT in 50% and 95%

ethanol at 40°C, and one antioxidant at two different temperature such as PG in 95% ethanol at 40°C and 20°C had not the same release behavior confirming the existence of 4 mentioned factors and additionally the simulant effect. If one suppose that all of the PLA films containing a common antioxidant had the same structures and the same polymer-antioxidant interactions, so simulant characteristics such as polarity, antioxidant solubility in simulant, and temperature are the major influencing factors for antioxidants release form PLA.

4- Conclusion

The release rates of some natural and synthetic antioxidants from PLA to food simulating solvents at 20°C and 40°C were studied. AP was completely degraded during PLA film preparation and did not show any release in none of the simulants. In general, main factors affecting antioxidant release form PLA into ethanolic simulants include: simulant polarity, antioxidant solubility in simulant, film thickness, temperature, agitation, antioxidant molecular weight and polymer degradation. The high polarity of 10% ethanol acted as a barrier and prevented the release of AT, BHA, BHT and TBHQ at 20°C, but increased molecular mobility of BHA and TBHQ provided at 40°C overcame this barrier. TBHQ was decomposed to TBBQ and other quinone derivatives in all simulants at 40°C and at weaker degree in 95% and 50% ethanol at 20°C; further studies needed to investigate the nature of these compounds and their formation mechanisms. Some neoformed compound also was detected from AT in 50% ethanol at 40°C with more hydrophobic characteristics which demands more research studies. The various velocities of antioxidants release from PLA and their final released concentration to simulants described by D and K coefficients show the possibility of PLA being as a good carrier for antioxidants to continually control food oxidation reactions. The D and K coefficients for three food simulants and two different temperatures can be used for diverse foodstuffs which are subjected to oxidation. So, PLA-antioxidant active packaging for various foodstuffs can be practically achieved by using the results of this study and also the supplementary data for other antioxidants involving their synergetic effects.

5- Acknowledgment

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Chapitre 3.5

Relargage d'Antioxydants Phénoliques

Synthétiques à Partir d'un Film de PLA Obtenu

par Extrusion

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Résumé

Des films d'acide poly lactique (PLA) contenant 2% d'antioxydants phénoliques synthétiques (APS), comme le BHA, BHT, PG et TBHQ ont été extrudés par une extrudeuse pilote. Le relargage de l'APS à partir des films du PLA-ASP dans 10%, 50% et 95% d'éthanol à 20°C et 40°C a été étudié par la mesure régulière des antioxydants libérés dans les aliments simulés par HPLC. La diffusion des APS dans le PLA en contact avec des aliments simulés suit un comportement Fickien avec une valeur de coefficient de diffusion (D) entre 10^{-8} et 10^{-11} cm^2s^{-1} avec 0-100% de la libération. Une libération plus rapide et plus élevée des APS a eu lieu à 40°C qu'à 20°C conformément à la loi d'Arrhenius. Le PG et le BHT avaient respectivement le taux de libération plus élevé et le plus bas dans tous les simulants en raison de leurs différentes propriétés moléculaires (polarité, Log P, volume moléculaire). Enfin, la libération totale et rapide des ASP dans 95% d'éthanol à 40°C ainsi que les petites quantités de APS libérées dans 10% d'éthanol à 40°C et 20°C permettent d'envisager l'utilisation du PLA-ASP comme une emballage actif pour les huiles, les aliments gras et certains produits laitiers, sensibles à l'oxydation.

Chapter 3.5

Release of Synthetic Phenolic Antioxidants from Extruded PLA

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Abstract

Poly lactic acid (PLA) and PLA containing 2% synthetic phenolic antioxidants (SPA) such as BHA, BHT, PG and TBHQ were extruded with a pilot-plant cast-extruder. SPA's release from PLA-SPA films into 10%, 50% and 95% ethanol at 20°C and 40°C was investigated via regular measurement of released antioxidant into simulant by HPLC system. Diffusion of the SPAs from PLA to simulants showed a Fick's behavior with diffusion coefficient (D) value between 10^{-8} and 10^{-11} cm^2s^{-1} with 0-100% of release. Faster and higher release of SPAs occurred at 40°C according to Arrhenius law. PG and BHT had respectively the highest and lowest release rate in all simulants due to their different molecular properties such as polarity, Log P and molecular volume. Finally, total and fast release of SPA into 95% ethanol at 40°C and small amounts of released SPAs into 10% ethanol at 40°C and 20°C suggests using PLA-SPA active packaging for oils, oily foods and some milk products which are highly sensitive to oxidation.

1- Introduction

Passive packaging has four main functions including containment, protection, convenience, and communication while active packaging provides active protection by continually releasing active compounds such as antioxidants, antimicrobials, enzymes, flavors and nutraceuticals to package content. Active packaging as an innovative concept and due to its interaction with the package, the food and the environment, generates longer shelf-life foods, higher protection of flavors and lower usage of additives and preservatives in food formulations while maintaining the quality of product (Lopez-Rubio & Lagaron, 2011; Singh, Wani, & Saengerlaub, 2011). Direct antioxidants addition to food formulation and dipping food in antioxidants solution are the common way for protecting food lipids oxidation. The most applied synthetic phenolic antioxidants (SPAs) in food products include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), tert-butylhydroquinone (TBHQ) (Wanasundara & Shahidi, 2005). Limitations of direct addition of antioxidants to foods include specific limit of activity due to their participation in complex reactions in food systems, and lack of selectivity to target the food surface where most oxidative reactions occur (Gemili, Yemenicioglu, & Altinkaya, 2010). Among these SPAs the release of BHA and BHT from some polymers into foods and food simulants has been investigated. Briefly, these release studies include BHT from HDPE to food simulants (Till, Ehntholt, Reid, Schwartz, Sidman, Schwoppe, et al., 1982); BHT from LDPE to fatty food simulants (Han & Krochta, 2007) and oatmeal (Wessling, Nielsen, & Giacini, 2001); BHA and BHT from HDPE, LDPE and PP, PVC and PET (Dopico-Garcia, Lopez-Vilarino, & Gonzalez-Rodriguez, 2007), BHT from LDPE to Asadero chesse (Soto-Cantu, Graciano-Verdugo, Peralta, Islas-Rubio, Gonzalez-Cordova, Gonzalez-Leon, et al., 2008); BHT from poly(lactide-co-glycolide) to dry milk products and food simulants (Van Aardt, Duncan, Marcy, Long, O'Keefe, & Sims, 2007); BHA and BHT from multilayer co-extruded films made of HDPE, EVOH and LDPE to milk powder (Granda-Restrepo, Peralta, Troncoso-Rojas, & Soto-Valdez, 2009).

Recently, PLA has attracted many research studies in various fields such as drug delivery systems, tissue engineering and food packaging due to its high applicability, biodegradability

and significant characteristics (Jamshidian, Tehrany, Imran, Jacquot, & Desobry, 2010). As PLA has a high permeability to oxygen meaning that food products susceptible to oxidation will be exposed to oxygen when contained in PLA packaging. Addition of antioxidants to the polymer so that they can be desirably released to food is an indirect way to compensate the entered oxygen. The release of antioxidants varies based on polymer, antioxidant and product characteristics and environment conditions, for example the release rate is slow when release is governed by diffusion in the polymer matrix, and it is fast when governed by swelling or dissolution (Lopez-Rubio & Lagaron, 2011). As the first step, the release of antioxidants should be investigated from virgin extruded PLA and then their release if needed be improved by prevalent methods such as blending or addition of release retarding agents. To our knowledge, PLA-antioxidant release studies are limited to two studies including release of α -tocopherol, BHA and BHT from solvent-cast poly(lactide-co-glycolide) or PLGA in water and oil at 4 and 25°C (Van Aardt, Duncan, Marcy, Long, O'Keefe, & Sims, 2007) and release of α -tocopherol from extruded PLA in pure ethanol at 13, 23, 33 and 43°C (Manzanarez-Lopez, Soto-Valdez, Auras, & Peralta, 2011). The diffusion and partition coefficients of α -tocopherol were just calculated in the latter study.

Release is the result of the diffusion and equilibrium process involving the transfer of low molecular mass compounds from packaging into a food or food simulant. The migrants diffuse through the amorphous portion of the polymer matrix toward the interface where they are partitioned between the two media until their chemical potential values in both the polymer and the food reach the equilibrium. So, release is the result of diffusion, dissolution and equilibrium process and often described by Fick's second law (Crank, 1975; Soto-Cantu, et al., 2008). The release of SPAs from extruded PLA has not been well studied and was the subject of this work because of their common uses in food products. The aim of this study was to characterize the release behavior and calculate the release parameters (Diffusion and Partition coefficients) of common applied synthetic antioxidants in foods including BHA, BHT, PG and TBHQ from extruded PLA film into ethanolic simulants. Antioxidants release was studied in three food simulating liquids (10%, 50% and 95% ethanol in water) and at two temperatures (20°C and 40°C).

2- Materials and Methods

2-1- Materials

Poly (lactic acid) (PLA), 2002 D, was purchased in pellet form from Natureworks[®] Co., Minnetonka (USA); BHA, BHT, PG and TBHQ from Sigma-Aldrich (France), 2-*tert*-butyl-1,4-benzoquinone (TBBQ) from Sigma-Aldrich (USA), Chloroform D (99.8% CDCL₃) from Euriso-Top (France), chloroform, acetonitrile, and trifluoroacetic acid (TFA) from VWR international (France). All solvents provided as HPLC grade.

2-2- Film preparation method (Compounding, pelletizing, and film casting)

PLA-antioxidant films were prepared using cast extruder. For each PLA-antioxidant film, 2% w/w of PLA, antioxidant was added and then all were generously compounded using twin screw extruder (ZSK 32 MCC, Werner & Pfleiderer, Germany) and pelletized using a underwater pelletizer (Gala Map 5, Gala industries, USA). All PLA pellets were dried at 35°C for 48 h before extrusion film casting. A single screw film casting extruder (Mapre, Gauder, Belgium) equipped with 20 cm wide slit casting die used to make films. The extruder equipped with a single screw with $D = 33$ mm, $L/D = 33:1$, where D and L are diameter and length of the screw, respectively. The barrel had three heating zones and a 11 Kw motor turning the screw. The temperatures of the six extruder heating zones were set at 150, 160, 170, 175, 180 and 190°C, respectively. The screw speed for the samples was 50 rpm. An adapter was installed ahead of the barrel and its temperature was set at 190°C. Five heater cartridges, one thermocouple and one PLC controller were used to control the die temperature. The die temperature was fixed at 190°C. The chill roll was placed in 5 cm from the die and its temperature was kept at 20°C by temperature controller. The film was quenched by a chill roll and was transported to a pulling station using a nip roll. The speed of chill and nip rolls were controlled separately from the extruder using a dial and digital display. Finally, all extruded films were wound by an electric film winder at the speed of 3.5 mmin^{-1} . All extruded films exhibited a homogeneous distribution of antioxidants with no accumulation at the film surface. Film thickness was measured using mechanical micrometer

(Mitutoyo, Mitutoyo Corporation, Japan) according to ASTM D 374-99 and presented the average of eight measurements.

2-3- Quantification of antioxidants in PLA films

The amounts of antioxidants in PLA films after drying were measured by nuclear magnetic resonance spectroscopy (NMR). ¹H NMR data shifts as ppm were collected at 600.13 MHz on an Avance III 600 spectrometer (Bruker, Germany) at 298°K. PLA films were dissolved in CDCL₃ and antioxidants amount were quantified by comparing the peak area of each antioxidant in a sample with the one of the corresponding peak of the standard at a known concentration. Standard precautions regarding quantization in NMR were taken including identical spectrometers settings, repetition time between two consecutive scans long enough in comparison with relaxation times. All tests were accomplished in triplicate.

2-4- Release tests

To avoid that testing has to be performed with all (potential) food types; the European unions (EU) has defined some simulants that can be used (Commission Directive 2007/19/EC, 2007). These food simulants are as follows:

- . Simulant A: water, mimicking aqueous foodstuffs.
- . Simulant B: 3% acetic acid, mimicking acidic foodstuffs with pH <4.5.
- . Simulant C: 10% ethanol, mimicking alcoholic foodstuffs;
- . Simulant D: Olive oil or another approved oil, mimicking fatty foodstuffs.
- . 50% ethanol: mimicking high alcohol containing products and milk and some dairy products

Sometimes, it is not possible to use olive oil as a food simulant because the oil is absorbed too much by the sample or because the migrating substance reacts with the oil components. In such cases, the EU has assigned substitute simulants for simulant D. The simulants assigned are 95% ethanol and isooctane (Verrart, 2010). As the applied antioxidants in this study had hydrophobic characteristics, we supposed a zero release of antioxidants into simulants A and B and did not use them in our release study.

Pre-weighed PLA films containing antioxidants (6cm×1.5cm×~160µm) were placed in 10 mL-vials (Agilent, USA) and then 10 mL of each corresponding simulant including 95% ethanol, 50% ethanol, and 10% ethanol in distilled water were added to film samples somehow completely immersed in simulants. The physico-chemical properties of applied simulants are summarized in Table 1. Aluminum caps equipped with Teflon septum were then tightly sealed by special crimper (CR-20, Chromacol, UK). The vials containing film samples were placed in incubator (Memert, Germany) for being at 40°C and in dark thermostat room for 20°C. The vials were shook manually two times each day to minimize mass-transfer resistance of antioxidants from the film. This release test was accomplished in duplicate.

Table 1- Physicochemical properties of ethanolic simulants.

	pH	Polarity*
Ethanol 10%	6.27±0.01 ^a	14.40
Ethanol 50%	6.42±0.02 ^b	10.82
Ethanol 95%	8.14±0.01 ^c	8.94

* Hansen polarity, data obtained from Molecular Modeling Pro ⁵.

Significant difference ($P \leq 0.05$) is represented by different alphabetic letters.

2-5- Measurement of released antioxidants in simulants

Released antioxidants were measured at regular intervals by direct injection of each simulant to HPLC column. HPLC measurements were performed by a Shimadzu (Japan) equipped with a quaternary pump (LC-20AD), an auto-injector (SIL-20AC HT), a UV-vis photodiode array detector (SPD-M20A), a column oven (CTO-20AC) and Labsolution data software. PLA film samples containing BHA, BHT, PG and TBHQ were analyzed using isocratic mode of acetonitrile-0.1% TFA (90%) and H₂O-0.1% TFA at pH 3.0 (10%) at a flow rate of 0.5

mL.min⁻¹. The simulants (5-50 µl) were injected onto a Alltima™ HP C18, 3µm (150×3 mm i.d.) column (GRACE, Deerfield, IL, USA) protected by a 2.1×7.5 mm Alltech guard C18 precolumn (GRACE, Deerfield, IL, USA) at 25°C. Each antioxidant was indentified by comparing retention times and photodiode array spectra in the range of 200-800 nm. A stock standard solution containing about 0.035 mgmL⁻¹ of each antioxidant was prepared in methanol and stored at 4°C for 24 h. Purity of each peak was checked so as to exclude any contribution from interfering peaks. Detection of BHA, BHT, PG and TBHQ was performed at 280 nm. Quantification was then carried out by comparing the peak area of each antioxidant in a sample with that of the corresponding peak of the standard. Limit of detection (LOD) and limit of quantification (LOQ) of antioxidants by HPLC system are listed in Table 2. The linearity of the determination method was measured by injecting standard solution of different concentrations (0.1-5 mgmL⁻¹, 9 points). Repeatability and reproducibility of the HPLC analysis were evaluated by analyzing five replicates of standard solutions in three different days.

Table 2- Antioxidants LOD^a, LOQ^b and recoveries in applied simulants.

	RT (min)	LOD (mg)	LOQ (mg)	95% ethanol	50% ethanol	10% ethanol
BHA	2.0	3.7×10 ⁻⁸	3.7×10 ⁻⁷	96.1±0.8	94.7±0.9	92.3±1.5
BHT	4.4	8.0×10 ⁻⁸	8.0×10 ⁻⁷	97.7±0.8	93.7±1.0	90.1±1.4
PG	1.5	1.0×10 ⁻⁹	1.5×10 ⁻⁸	98.3±0.9	96.6±1.0	95.4±1.4
TBHQ	1.7	1.1×10 ⁻⁹	1.7×10 ⁻⁸	99.2±1.0	98.5±0.9	89.8±1.2

a= limit of detection and b= limit of quantification.

Recovery tests were accomplished by measurement of antioxidants prepared in the simulants. Linear regression analysis showed a linear relationship between peak area and concentration of standard samples (0.1-5 mgmL⁻¹, triplicate 9 points). The correlation coefficient (R²) was 1

for BHT, PG and TBHQ and 0.999 for BHA. The obtained relative standard deviation (RSD) for repeatability and reproducibility were 0.18% and 0.44% (BHA), 0.12% and 0.11% (BHT), 0.28% and 0.61% (PG) and, 1.35% and 2.96 % (TBHQ), respectively. Recovery experiments were accomplished by spiking known antioxidants standard concentrations within three applied simulants. Antioxidants average recoveries and retention times (RT) are presented in Table 2.

At the end of each working day, the whole chromatographic system was rinsed with water/methanol/acetonitrile 50:25:25 (v/v/v) for 1 h.

2-6- Calculation of diffusion and partition coefficients

The apparent diffusion coefficients (D) of antioxidants were determined from the release versus time data, which was fitted to Fick's second law for an infinite slab in contact with an infinite volume of solvent (equation 1, Crank, 1975).

$$\text{Eq. 1} \quad \frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp \left[-\frac{(2n+1)^2 \pi^2}{L^2} D t \right]$$

Where M_t/M_∞ is the concentration of antioxidant diffused at time t , divided by the concentration of antioxidant diffused at equilibrium and L is the thickness of the film.

For short times ($M_t/M_\infty < 2/3$), equation 1 can be simplified to equation 2 and D can be determined with equation 3.

$$\text{Eq. 2} \quad M_t/M_\infty = 4 (D t / 4L^2 \pi)^{1/2}$$

$$\text{Eq. 3} \quad D = (S.L/2)^2 \pi$$

Where, S is the slope of a plot representing M_t/M_∞ against $t^{1/2}$.

The partition coefficient of a migrant is defined as the ratio of migrant equilibrium concentration in the polymeric material, C_p , to its equilibrium concentration, in the food phase, C_s (Eq. 4) (Tehrany & Desobry, 2004).

Eq. 4
$$K = C_p/C_s$$

The remained amounts of antioxidants in PLA films after equilibration were measured by NMR.

2-7- Thermal properties

Thermal analysis for measurement of transition temperatures including glass transition temperature (T_g), melting temperature (T_m), enthalpies of fusion (ΔH_f) and crystallization (ΔH_c) was carried out with a differential scan calorimeter (NETZSCH, 204F1 Phonex, Germany). Specimens approximately weighing 3-5 mg were sealed in an aluminum pan and heated under protective nitrogen (40 mLmin⁻¹) at the rate of 10 °Cmin⁻¹ from 5 to 180°C then cooled to 5°C and reheated to 180°C with the same cooling-heating rates.

T_g , T_c and T_m were determined from the obtained thermogram. The degree of crystallinity was evaluated according to equation 5:

Eq 5.
$$\% \text{ Crystallinity of PLA} = [(\Delta H_m - \Delta H_c)/\Delta H_f]$$

Where ΔH_m is the enthalpy of fusion; ΔH_c is the enthalpy of crystallization and ΔH_f is the enthalpy of melting of 100% crystalline PLA (93.6 J/g) (Arnoult, Dargent, & Mano, 2007).

2-8- Statistical analysis

The data were analyzed by analysis of variance (ANOVA) using StatGraphics (version 16, StatPoint Technologies, Inc., Warrenton, VA, USA) and differences among mean values were processed by the Student-Newman-Keuls multiple range test. Multiple comparisons were performed through 95% least significant difference intervals ($P \leq 0.05$).

3- Results and discussion

3-1- Initial antioxidant concentration in PLA

NMR showed some antioxidants loss during film manufacturing due to thermal effect of extrusion. The maximum loss was observed for TBHQ (45%) while BHA had 14.3%, BHT 10.5% and PG 18.8% of loss. Thermal decomposition of SPAs at 185°C and atmospheric pressure was studied by Hamam and Nawar (1991); their results showed that TBHQ had the highest loss followed by BHA due to thermal decomposition. Obviously, the combined effects of high temperature and pressure in extrusion process result in different chemical decomposition rate of a given antioxidant. It should be pointed out that these antioxidants possess varying degree of volatility and thermal stability leading various loss rates and also diverse neoformed compounds. One of the major neoformed compound from TBHQ decomposition is *2-tert-butyl-1,4-benzoquinone* (TBBQ); this compound was detected in PLA-TBHQ film before release test start and traced during release in simulated foods. No other neoformed compounds from antioxidants decomposition due to extrusion process were detected in simulants.

3-2- Release levels at 40°C

3-2-1- Antioxidants release into 95% ethanol

The 95% ethanol usually used as a fatty simulant for fats, oil and fatty foods due to its similar hydrophobicity. Ethanol is fully miscible in water, and at the same time it provides a more hydrophobic environment. Since all applied SPAs in this study have hydrophobic characteristics, their rapid and high release levels were anticipated. For a better understanding of antioxidants release behaviors, all the release graphs are presented as the ratio of released antioxidant concentration (M_t) divided to initial antioxidant concentration in PLA film (M_0). The release of BHA, BHT, PG and TBHQ into 95% ethanol, as the ratio of released antioxidant to its initial concentration in PLA (M_t/M_0), is presented in Fig 1.

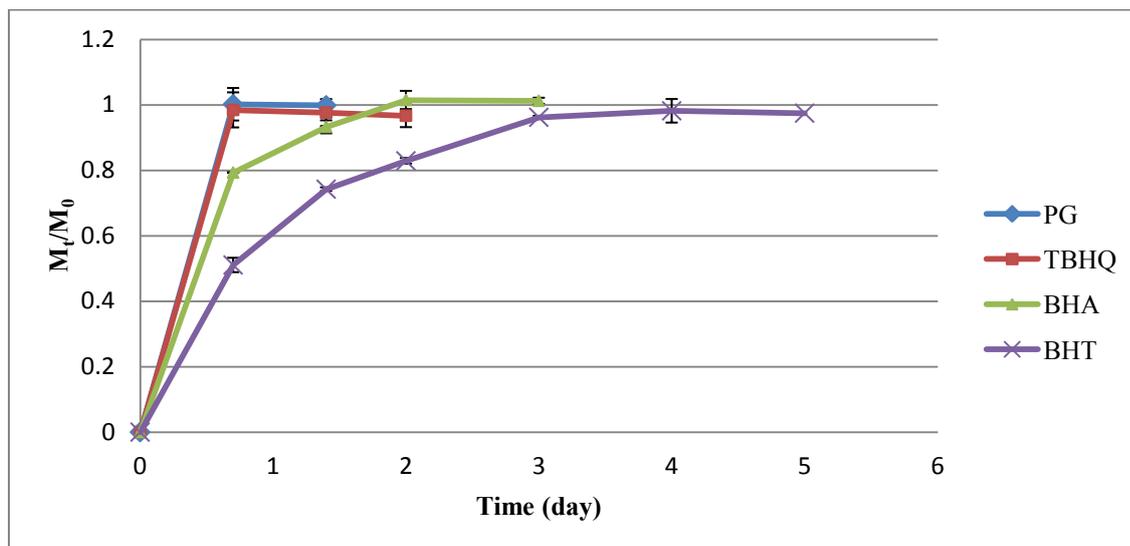


Fig 1- Antioxidants release rate (M_t/M_0) into 95% ethanol at 40°C.

PG and TBHQ rapidly released to simulant with approximately the same velocity; they are fully liberated to simulant after 17h. BHA with a slower velocity reached its maximum release after 48 h. BHT release to simulant occurred at the minimum velocity and reached its maximum level after 4 days. These diverse release behaviors were attributed to the antioxidant intrinsic properties including molecular weight, molecular volume, polarity, Log P and also extrinsic factors such as the degree of antioxidant involvement in polymer matrix and the mobility across polymer chains towards simulant. Antioxidants polarity (Table 3) showed that PG and TBHQ have the highest value while BHA and BHT have the same one; but the release rate of BHA is faster than BHT. One explanation is BHT higher molecular weight and molecular volume (because of two $-C(CH_3)_3$ groups) than BHA decelerating BHT movement across polymer matrix and consequently slower velocity of release. Han and Krochta (2007) reported a rapid release of BHT from extruded LDPE into 95% ethanol; BHT total release occurred after 1 week at 4°C and after 1 day at 20°C. TBBQ was rapidly detected into simulant as well as TBHQ; it should be pointed out that all detected TBBQ was not originated from oxidized TBHQ during extrusion process because the released TBHQ was also quickly oxidized to TBBQ. We tested the amount of TBBQ in a freshly prepared TBHQ solution kept at 40°C and observed that TBBQ was formed at the first hours and continued to increase correlated to TBHQ reduction.

As seen in Fig 2, TBHQ oxidation rate of fresh solution at the first hours of study is rapid and then begun to decelerate and finally stabilized. The rate of TBBQ formation has the same tendency as TBHQ reduction and stabilized after 8.5 hours at 40°C. The concentration sum of TBHQ and TBBQ at first hours of study was equal to initial concentration of TBHQ but after TBBQ stabilization, the TBHQ reduction continued and the sum of TBHQ and TBBQ was less than initial amount of TBHQ. It means that TBHQ was decomposed to form other compounds, as confirmed by small peaks detection by HPLC after 23th hour. We did not characterize these neoformed compounds structures and further studies are needed to precisely investigate TBHQ decomposition in 95% ethanol. As TBBQ has no antioxidant activity, we considered it as TBHQ loss and just took into account the unaltered TBHQ in the simulant.

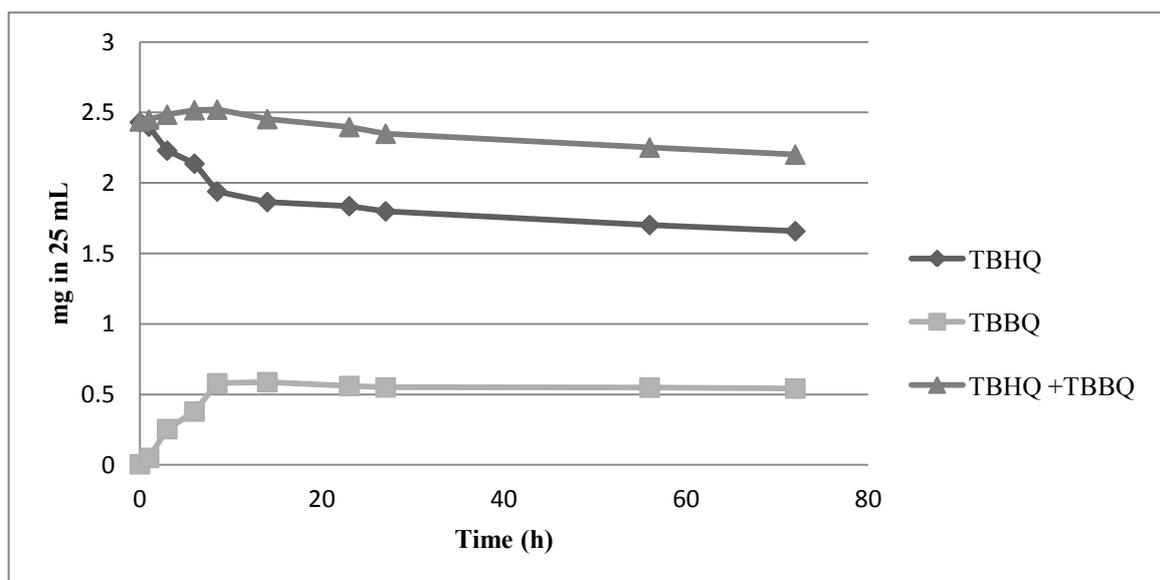
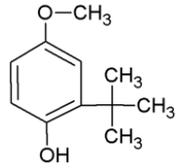
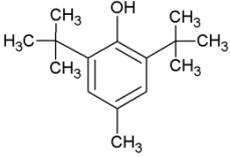
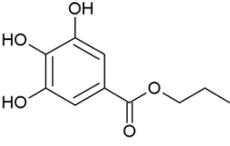
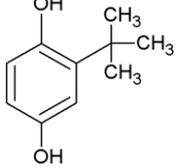


Fig 2- TBHQ decomposition with TBBQ formation of a pure solution of TBHQ (0.1 mgmL⁻¹) in 95% ethanol at 40°C.

As all initial amounts of SPAs were fully released to the simulant, there is no residual entrapped or bonded antioxidant in PLA matrix at the end of the experiment.

Table 3- Antioxydants physicochemical properties.

	Mw	Flash point(°C)	Molecular volume (cm ³ mol)	Polarity ^{1a}	Log P ^{1b}	Chemical structure
BHA	180.25	130	182.4	4.0	3.4	
BHT	220.54	118	255.0	4.1	6.6	
PG	212.20	187	152.7	9.2	1.2	
TBHQ	166.22	171	162.0	4.8	3.0	

Data obtained from Molecular Modeling Pro⁵. a= Hansen polarity and b= Log P or lipophilicity is the logarithm of the ratio of un-ionized antioxidant concentrations in octanol and water.

3-2-2- Antioxydants release into 50% ethanol

The 50% ethanol is recommended by the European commission as simulant for some dairy products. The M_t/M_0 of BHA, BHT, PG and TBHQ are presented in Fig 3. The release

kinetics (PG> TBHQ> BHA> BHT) in 95% ethanol was changed a little in 50% ethanol (PG> BHA> TBHQ> BHT). This was occurred because of TBHQ oxidation to TBBQ reducing the real amount of TBHQ measured in simulant. Additionally, the total release of BHT did not occurred after 60 days showing insufficient affinity of 50% ethanol for total extraction of BHT compared to 95% ethanol. The highest value of Log P (highest lipophilicity) for BHT determines the minimum affinity of 50% ethanol for BHT among other antioxidants. PG release kinetic was significantly higher than others; PG and BHA reached to their total release amounts after 18 and 48 days, respectively. BHT and TBHQ released gradually to simulant and remained at 60% and 40% of their initial concentrations, respectively. The same explanation for lower release rate of BHT into 95% ethanol can be also applied for 50% ethanol As discussed above no chemical bonding exists between PLA and the antioxidants, so it can be concluded that the condition of 50% ethanol at 40°C led to slow extraction of BHT and TBHQ from PLA films.

The rate of TBHQ and TBBQ release from PLA into 50% ethanol and also TBBQ formation from released TBHQ were slower than in 95% ethanol. It can be mentioned that the 95% ethanol rapidly extracted all TBHQ along with some TBBQ (formed during extrusion process); then the released TBHQ begun to be oxidized to TBBQ. The measured amount of TBBQ was one tenth of amount measured in 95% ethanol explaining the potential effect of ethanol concentration on TBBQ formation. TBBQ formation in 50% ethanol from a pure TBHQ solution kept at 40°C was slower than in 95% ethanol (data not shown here) approving the effect of ethanol content on TBHQ oxidation. It seems this effect is attributed to higher solubility of TBHQ and TBBQ in 95% ethanol.

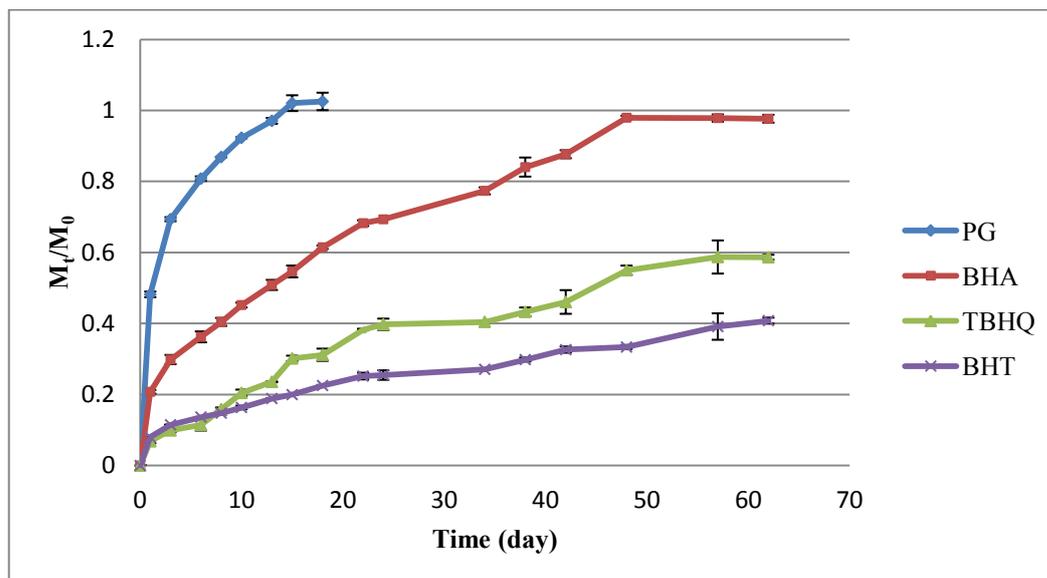


Fig 3- Antioxidants release rate (M_t/M_0) into 50% ethanol at 40°C.

3-2-3- Antioxidants release into 10% ethanol

The EU Commission specified 10% ethanol as the simulant for alcoholic foods while FDA expanded its application to aqueous, acidic and alcoholic (up to 15%) foods (CFR, 1995). Among the four SPAs, only PG and BHA slightly released to 10% ethanol. The M_t/M_0 of BHA and PG are presented in Fig 4. Similarly to 95% and 50% ethanol, PG released more rapidly than BHA due to its higher polarity and lower Log P. BHA release occurred due to its low molecular volume permitting its movement through polymer chains with higher molecular mobility provided by the temperature of 40°C. TBHQ showed a small release into 10% ethanol while small amounts of TBBQ detected as well. However, it seems that lower Log P and molecular volume of BHA and PG somehow permit their release into highly polar simulants. Lack of apolar additives release from apolar polymers, such as low density polyethylene (LDPE), polypropylene (PP) and polystyrene (PS), in contact with more polar food simulants, such as water or 3% acetic acid have been reported. Higher release rates are, therefore, often found for fatty, for example olive oil, 95% ethanol or isooctane, than for aqueous food simulants (Linssen, Reitsma, & Cozijnsen, 1998; Riquet & Feigenbaum, 1997; Till, et al., 1982).

As will be discussed in next part, some PLA films were variably hydrolyzed in some simulants. In hydrolysis process the amorphous phase of PLA film is hydrolyzed and consequently the crystalline part or crystallinity is increased. The maximum rate of hydrolysis was observed for PLA films in contact with 10% ethanol at 40°C. So, another decelerating factor observed in 10% ethanol at 40°C was the increase of PLA crystallinity during release study which resulted more tortuosity in polymer matrix. More tortuous the polymer much slower the transfer of migrant occurs because the migrant has to spend more time in polymer matrix to pass longer ways made by new formed crystals. Despite the more tortuous matrix, PLA-BHA and PLA-PG had more fragile structures after release study approving their higher hydrolysis than PLA-BHT and PLA-TBHQ. PLA hydrolysis facilitates antioxidant liberation into simulants, so it can be supposed that the PLA hydrolysis also intensified the release of BHA and PG into 10% ethanol at 40°C.

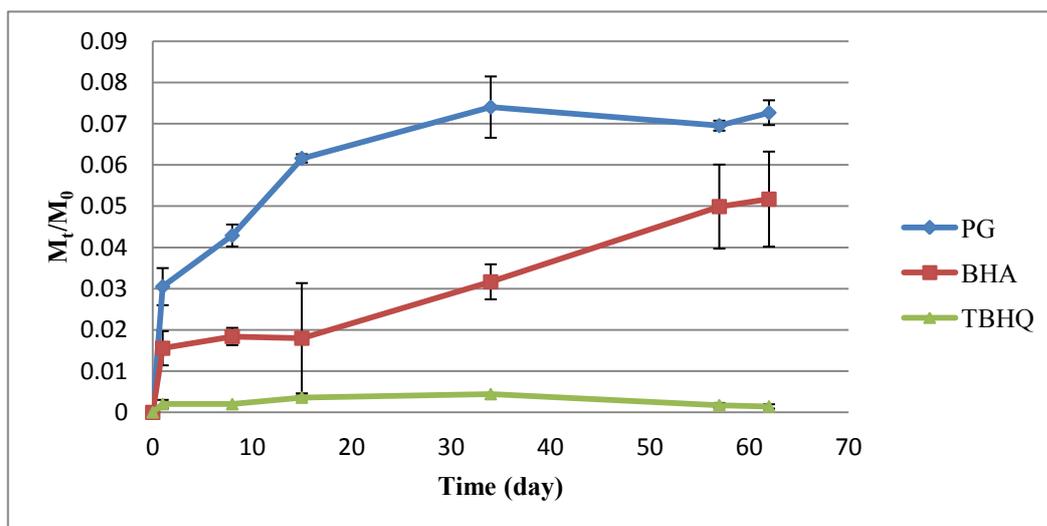


Fig 4- Antioxidants release rate (M_t/M_0) into 10% ethanol at 40°C.

3-2-4- PLA degradation during release test

The majority of PLA films did not keep their initial forms and transparencies during release kinetics; some of them turned fragile and opaque and some became white-opaque. The higher the temperature and the ethanol content the more opaque the films became, which could be attributed to an increase of crystallinity of the films due to hydrolysis of the amorphous regions. At 40°C, all PLA films in contact with 50% ethanol turned opaque and fragile, the PLA-BHA and PLA-PG films in contact with 10% ethanol turned white opaque and fragile while PLA-BHT and PLA-TBHQ turned white opaque but remained rigid. At 20°C, unless PLA-PG in contact with 50% ethanol, all other PLA films in contact with 95% and 50% ethanol remained transparent and rigid. This different changes can be attributed to the degree of hydrolysis occurred; in hydrolysis process the amorphous phase decomposed resulting in the increase of crystalline phase and consequently opacity of PLA film. The %crystallinity before and after release study approved the hydrolysis of amorphous phase; as shown in Fig 5 the amorphous phase of PLA-PG (B) disappeared after 60 day in contact with 10% ethanol at 40°C (C) while did not change in contact with 95% ethanol at 20°C (A). The 3.4% crystallinity of initial PLA-PG in contact with 10% ethanol at 40°C increased to 41.4% approving the hydrolysis of amorphous phase. PLA amorphous phase hydrolysis was also observed by others (Kale, Auras, & Singh, 2007); Manzanarez-López et al. 2011). The fragility of PLA films related to their degradation and the decrease of molecular weights. Random non-enzymatic chain scission of the ester linkages in the polymer backbone leads to a reduction in molecular weight and is accelerated by acids or bases, affected by temperature and moisture levels. As presented in Table 4, the minimum and maximum hydrolysis was observed for the films in contact with 10% ethanol at 20°C and 40°C, respectively. By comparing the PLA films in contact with 50% and 10% ethanol at 20°C and 40°C, it can be stated that the influence of ethanol on PLA hydrolysis at 20°C is noticeable while the hydrolysis at 40°C occurred at almost the same rate.

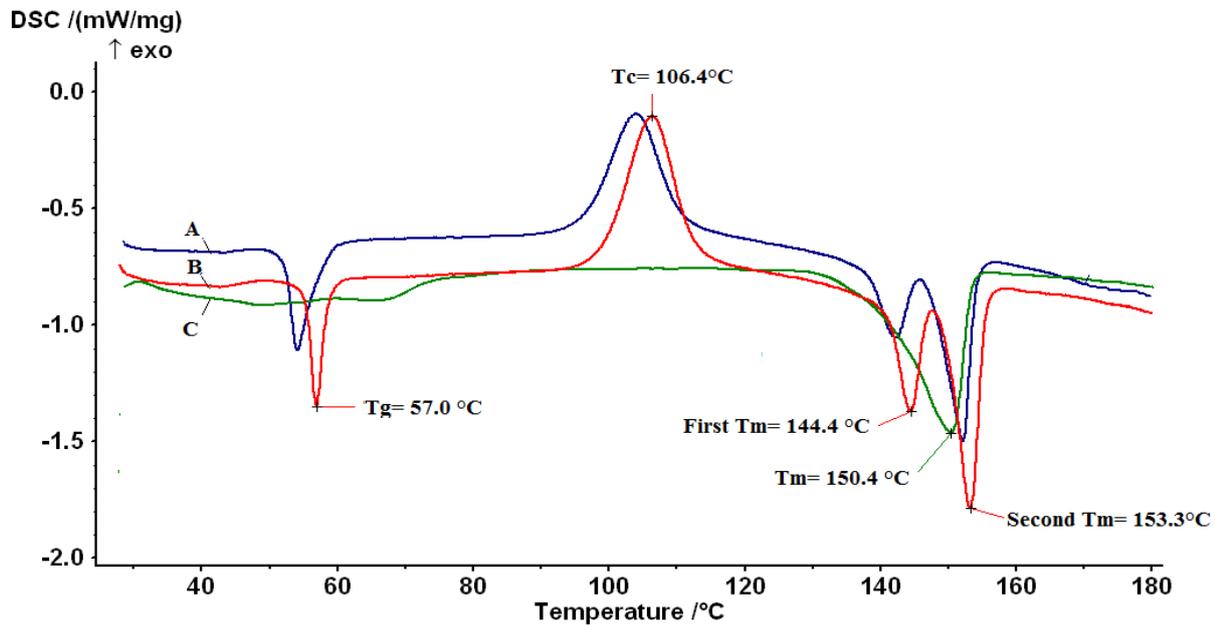


Fig 5- PLA amorphous phase hydrolysis during release study; A= PLA-PG into contact with 95% ethanol at 20°C for 60 days, B= PLA-PG before release study, C= PLA-PG into contact with 10% ethanol at 40°C for 60 days.

Table 4- Crystallinity of PLA-PG films before and after release study

	20 °C		40 °C	
	Appearance	%Cryst.	Appearance	%Cryst.
T ₀ ¹	Transparent	3.4±1.5 ^a	Transparent	3.4±1.5 ^a
95% ethanol	Semi-opaque	30.7±1.4 ^b	Semi-opaque	32.5±1.1 ^b
50% ethanol	Transparent	19.1±0.9 ^c	Semi-opaque, fragile	37.2±1.3 ^c
10% ethanol	Transparent	2.8±1.6 ^a	Opaque, highly fragile	41.2±0.8 ^c

T₀ is the PLA-PG film before release study. Significant difference (P≤0.05) is represented by different alphabetic letters.

3-3- Release levels at 20 °C

3-3-1- Antioxidants release into 95% ethanol

A reduced release rate of antioxidants was expected at 20°C, the M_t/M_0 of BHA, BHT, PG and TBHQ in 95% ethanol at 20°C is presented in Fig 6. The temperature-dependent diffusion of antioxidants through PLA matrix can be described according to Arrhenius equation:

Eq. 3
$$D = D_0 \cdot e^{-E/RT}$$

Where D is the diffusion coefficient of the antioxidant in the polymer, T is the absolute temperature, R is the general gas constant, E is the activation energy and D_0 is a constant. As been seen in Fig 6, the order of release (PG > TBHQ > BHA > BHT) did not change compared to 95% ethanol at 40°C. So, it can be stated that in one hand by reducing the temperature the molecular mobility of antioxidants in polymer matrix and also their solubility in simulant are decreased and in another hand less polymer chains expansion does not provide more voids and spaces for antioxidants transfer towards simulant. Additionally, except PG, other antioxidants did not reached to their total release after 60 days. BHA and BHT equilibrated at around 65% and 25% of their initial concentrations in PLA, respectively; as their solubility in 95% ethanol is much higher than their total release, so it can be concluded that their restricted molecular mobility and difficulty in movement through polymer chains limited their release at respected levels. The 95% ethanol extracted all amount of PG due to its high polarity value or in another word high affinity for 95% ethanol acted as a driving force and took out the PG from PLA. TBHQ and TBBQ release accompanied with TBHQ oxidation observed in 95% ethanol; TBHQ release continued to 66% of its initial concentration and then begun to decrease to 50% until 34th day and finally equilibrated to 55%. The reduction of temperature also affected the oxidation of TBHQ, the TBBQ amount formed from a pure solution of TBHQ at 20°C was around half of the amount formed at 40°C (data not shown here).

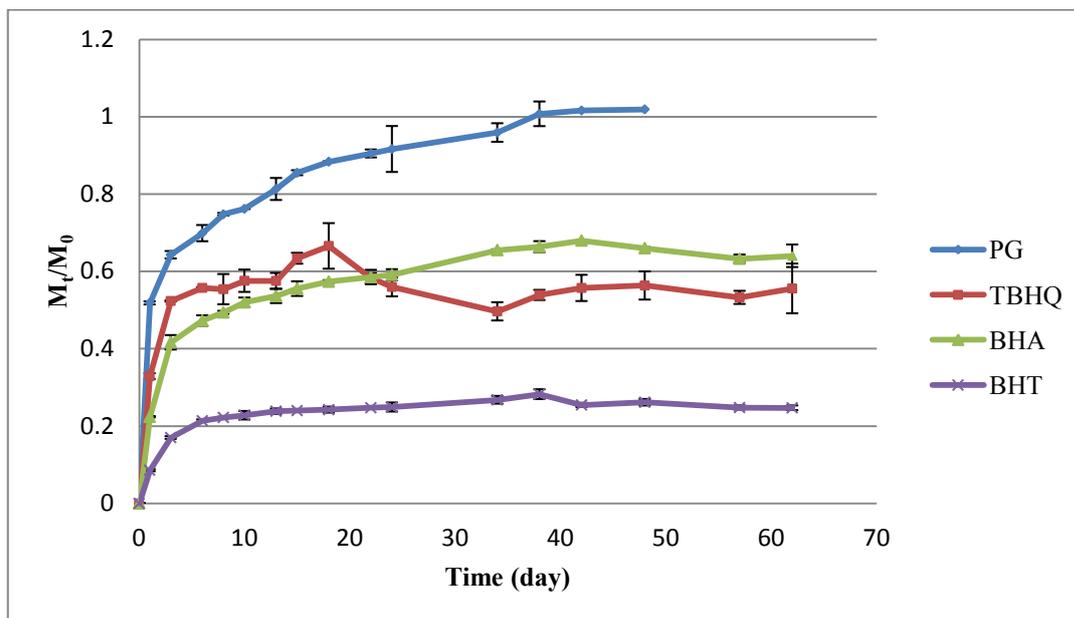


Fig 6- Antioxidants release rate (M_t/M_0) into 95% ethanol at 20°C.

3-3-2- Antioxidants release into 50% ethanol

The M_t/M_0 of BHA, BHT, PG and TBHQ in 50% ethanol at 20°C is presented in Fig 7. The order of antioxidant release was different from that occurred in 50% ethanol at 40°C matching the inverse order of Log P; this was due to lower rate of TBHQ oxidation to TBBQ. It seems that just BHT equilibrated at its released concentration after 60 days and release of other antioxidants tends to be continued. Log P and molecular volume are the main factors controlling the antioxidant release in 50% ethanol. TBBQ formation was around one tenth of that observed in 50% ethanol at 40°C and one fortieth of that in 95% ethanol showing the influence of temperature and ethanol concentration on TBHQ oxidation to TBBQ.

One important reason for the much higher release of antioxidants in 50% ethanol at 40°C rather than 20°C is the PLA hydrolysis occurred at 40°C facilitating the liberation of antioxidants into the simulant. As mentioned above PLA films in contact with 50% ethanol at 40°C had the most fragile structure approving their higher rate of hydrolysis. Therefore, not only the rate of penetration of ethanol could affect the diffusion of antioxidants but also the hydrolysis of the polymer. Both depend on temperature.

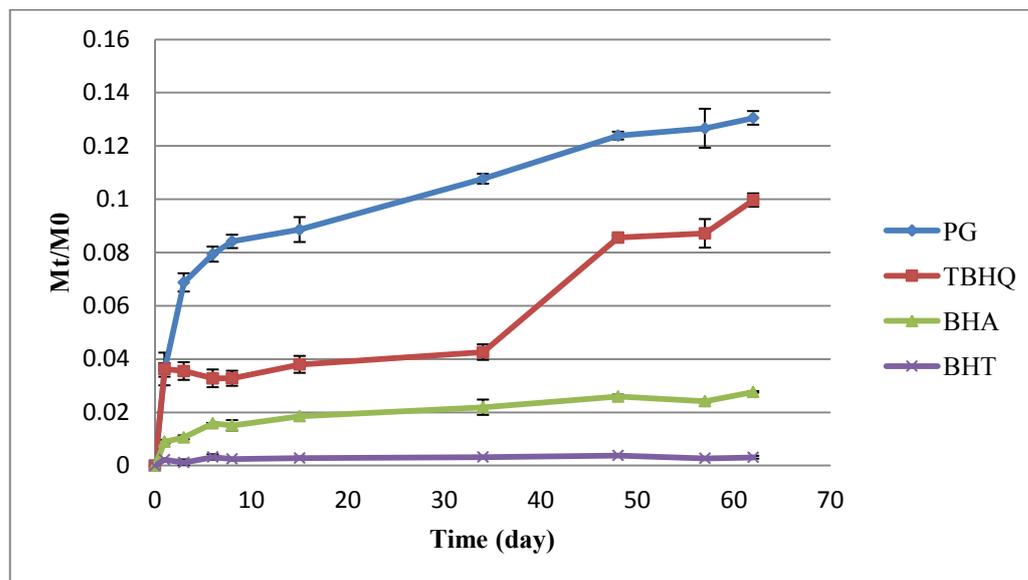


Fig 7- Antioxidants release rate (M_t/M_0) into 50% ethanol at 20°C.

3-3-3- Antioxidants release into 10% ethanol

No significant release of antioxidants was observed into 10% ethanol; trace concentrations of BHA, PG and TBHQ were detected in last days of study confirming the inefficiency of this system for antioxidants release. Weak simulant affinity, low molecular mobility and insufficient polymer expansion inhibited antioxidants release into 10% ethanol solution.

4- The D and K coefficients

D and K coefficients are good criteria in evaluating a migrant suitability for active packaging applications. D coefficients of studied antioxidants into food simulants are listed in Table 3. As seen, D coefficient was influenced by both the percent of ethanol in simulant and the temperature. For all antioxidants the highest value of D attributed to 95% ethanol at 40°C and PG had the highest D value in all conditions. Despite the influence of temperature on antioxidant diffusion, the order of magnitude of D coefficient for 95% ethanol at 40°C is higher one order of magnitude than 95% ethanol at 20°C meanwhile it has the same value in 50% ethanol at 40°C and 20°C showing the distinct effect of ethanol on antioxidant diffusion in polymer. Ethanol as an aggressive simulant entering in polymer matrix and facilitating the

diffusion of antioxidant both via providing more gaps and spaces in polymer matrix and also acting as an extractor for antioxidants. The influence of temperature can be observed in 10% ethanol where no release occurred at 20°C while BHA and PG as low volume molecules released at 40°C.

D coefficient of BHT was also studied for HDPE and LDPE in contact with food simulants. Till et al., (1982) found D coefficients of 6.5×10^{-13} and $8.6 \times 10^{-13} \text{ cm}^2 \text{ s}^{-1}$ for BHT in HDPE in contact with water and 8% ethanol at 40°C, respectively. (Schwope, Till, Ehntholt, Sidman, Whelan, Schwartz, et al., 1987) found D coefficients of 10^{-12} , 10^{-12} and 10^{-10} for BHT in LDPE in contact with orange juice, 8% ethanol and 50% ethanol at 49°C, respectively; the latter is consistent with the D coefficient of BHT in PLA in contact with 50% ethanol at 40°C.

Table 3- D coefficients ($\text{cm}^2 \text{ s}^{-1}$) of antioxidants in PLA film in contact with food simulants at 40°C and 20°C.

	<u>40°C</u>			<u>20°C</u>		
	<u>95%</u>	<u>50%</u>	<u>10%</u>	<u>95%</u>	<u>50%</u>	<u>10%</u>
BHA	5.99×10^{-9}	3.65×10^{-11}	1.77×10^{-11}	2.66×10^{-10}	5.74×10^{-11}	n.d ^a
BHT	2.41×10^{-9}	2.70×10^{-11}	n.d ^a	2.97×10^{-10}	2.72×10^{-11}	n.d ^a
PG	1.76×10^{-8}	1.02×10^{-10}	7.93×10^{-11}	3.09×10^{-9}	1.50×10^{-10}	n.d ^a
TBHQ	n.d ^b	n.d ^b	n.d ^b	n.d ^b	n.d ^b	n.d ^b

n.d= not determined; a: because the antioxidant did not reach to equilibrium and b: because of TBHQ decomposition and inability to measure its real value.

K coefficients of antioxidants are listed in Table 4. Calculation of K coefficient implies equilibration concentration both in film and simulant. As some antioxidant totally released

into simulants or their release seems to be continued after 60 day, so their K coefficients are not calculable. A higher value of K coefficient shows the tendency of antioxidant to remain in polymer which is not desirable for active packaging applications; additionally, the rapid total release of antioxidant into simulant conflict with its gradual release aimed to control oxidation. For example, the high value of K for BHT in 50% ethanol at 20°C states the unsuitability of this antioxidant in PLA active packaging for controlling oxidation a kind of cheese at 20°C since BHT would remain in polymer. However, D and K coefficients calculated on the basis of release in food simulants give a general idea about their release behavior and obviously these coefficients should be also obtained under real conditions of use.

Table 4- K coefficients of antioxidants in PLA and food simulants at 40°C and 20°C.

	<u>40°C</u>			<u>20°C</u>		
	<u>95%</u>	<u>50%</u>	<u>10%</u>	<u>95%</u>	<u>50%</u>	<u>10%</u>
BHA	n.d ^a	n.d ^a	16.9±4.4	0.5±0.1	31.6±0.4	n.d ^b
BHT	n.d ^a	1.3±0.1	n.d ^b	2.7±0.1	294±56	n.d ^b
PG	n.d ^a	n.d ^a	11.5±0.6	n.d ^a	6.0±0.2	n.d ^b
TBHQ	n.d ^c	n.d ^b				

n.d= Not determined, a: because all antioxidant amount released into simulant, b: the antioxidant did not reach to its equilibration until 60th day, c: TBHQ decomposition and inability to measure its real value .

5- Factors influencing antioxidants release from PLA to ethanolic simulants

The properties of migrant, polymer and food simulant and, the temperature determine the velocity and quantity of migrant release. Migrants pass through voids and other gaps between

the polymer molecules. Therefore, the release rate extensively depends on the size and shape of the migrants and on the size and number of the gaps. The latter will depend on polymer properties such as density, crystallinity and degree of crosslinking and branching (Helmroth, Rijk, Dekker, & Jongen, 2002). According to Limm and Hollifield (1996) several factors influence antioxidants diffusion through polymer including: (1) related-polymer factors such as polymer morphology, crystallinity, molecular weight distribution, density, and orientation which eventually determine sizes and shapes of microcavities and their distribution, (2) related-antioxidants factors such as their molecular size, shape, density, polarity, and solubility, (3) factors related to the interaction of a polymer and antioxidant such a plasticizing or antiplasticizing effect, and (4) factors related to temperature such as T_g and T_m of polymer. They did not consider the potential adsorption and/or penetration of simulant or food into polymer matrix. Manzanarez-López et al., (2010) concluded that ethanol acted as an aggressive solvent and can penetrate the PLA chains and promote the diffusion of α -tocopherol. (Mascheroni, Guillard, Nalin, Mora, & Piergiovanni, 2010) speculated that ethanol would play as a plasticizer for the PLA matrix and would open the structure creating void spaces favoring the polyphenols release. Absorption of certain organic solvents causes swelling of the polymer matrix, thereby enlarging the gaps and increasing additive release rates (Helmroth, Dekker, & Hankemeier, 2002).

In this study by using 4 phenolic antioxidants, 3 food simulants and 2 temperatures, one can observe the effects of all above mentioned factors on antioxidants release from PLA into simulants. As earlier discussed, antioxidants thermodynamic properties such as polarity and solubility influence their release rate due to interactions between polymer, antioxidant and food simulant. Briefly, faster release of PG than BHT into 95% ethanol at 40°C showed the effect of both antioxidant molecular volume and polarity; the total and faster release of BHA into 95% ethanol rather than 50% ethanol at 20°C showed the influence of simulant polarity and antioxidant solubility; higher temperature led to higher PLA crystallization in one hand that caused more tortuous matrix reducing the antioxidants release rate and in the other hand provided higher mobility and release of antioxidants. All these cases showed the importance of temperature, and antioxidants and simulants properties in antioxidants release process from extruded PLA. If one suppose that all of the PLA films containing the same antioxidant had

the same structures and the same polymer-antioxidant interactions, so the characteristics such as simulant polarity, antioxidant properties such as molecular volume, Log P and solubility in simulant, and temperature are the major influencing factors for antioxidants release from extruded PLA.

The high and fast release of SPAs into 95% ethanol at 40°C is not conformed with the aim of active packaging and further modification needed to decelerate their release for instance adding some retarding agents or blending with other polymers. Schaich, Obinata and Yam (2008) incorporated polypropylene into LLDPE (50:50) via smart blending and showed that the 83% release of α -tocopherol into ethanol 95% from pure LDPE decreased to 63% from blended film (Schaich, Obinata, & Yam, 2008). Siro et al. (2006) concluded that a controlled release of α -tocopherol from LDPE achieved by inclusion complexation with beta-cyclodextrin. They showed that the 85% release of α -tocopherol into 95% ethanol from extruded LDPE occurred in 400 h can be retarded to 2324 h by complexation of α -tocopherol with beta-cyclodextrin. (Siró, Fenyvesi, Szente, De Meulenaer, Devlieghere, Orgoványi, et al., 2006). Since small amount of antioxidants released into 10% ethanol at both 20°C and 40°C, it can be concluded that PLA-SPA active packaging is rather suitable for oils, oily foods and some dairy products.

6- Conclusion

The release study of 4 synthetic phenolic antioxidants (SPA) including BHA, BHT, PG and TBHQ into 3 food simulants including 95%, 50% and 10% ethanol at 20°C and 40°C was accomplished. Diffusion of SPAs from PLA films to simulants showed a Fick's behavior. PG had the highest and fastest rate of release in all simulants at 20°C and 40°C due to its higher polarity and lower Log P rather than other antioxidants. TBHQ decomposed to TBBQ both during extrusion process and release tests under all conditions. BHT had the lowest rate of release in all cases and completely retained in PLA in contact with 10% ethanol. As expected for apolar migrants, the D coefficient of antioxidants decreased by increased polarity of the simulant. In general, main factors affecting SPAs release from extruded PLA into ethanolic simulants include: antioxidant properties including its solubility in simulant, Log P, polarity,

molecular weight and molecular volume, simulant polarity, film thickness, temperature, agitation, and polymer degradation. The fast and total release of SPAs to 95% ethanol at 40°C shows the requirement of further studies to decelerate their release for instance using retarding agents in PLA matrix. Small release of SPAs into 10% ethanol showed the suitability of PLA-antioxidant active packaging for oils, oily foods and some milk products which are actually in danger of oxidation. Complementary studies using real foods in contact with PLA-antioxidant films needed to fulfill a better understanding of their release in real conditions.

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Chapitre 3.6

Modélisation du Relargage d'Antioxydants Présents dans le PLA en Contact Alimentaire

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Résumé

Les emballages actifs antioxydants sont prometteurs pour la protection des aliments. Dans cette étude, la relargage (diffusion et partage) de six antioxydants (α -tocophérol, palmitate d'ascorbyle, BHA, BHT, PG et TBHQ) à partir d'emballage actif à base du PLA a été étudiée dans les aliments simulants. Un modèle basé sur l'équation de diffusion de Fick a été utilisée pour calculer le coefficient de diffusion (D) des antioxydants à deux températures de 20°C et 40°C. Une relation quantitative structure-propriété (RQSP) a été élaborée pour calculer les valeurs des coefficients de diffusion et de partage à partir de descripteurs moléculaires. Nous avons développé une méthode simple d'estimation des coefficients de diffusion de 15 systèmes (PLA/antioxydant/aliment simulant) de diverses propriétés physiques et chimiques telles que polarité, force ionique, concentration molaire, électronégativité et rayons ioniques. Les descripteurs moléculaires ont été tirés de descripteurs électrostatiques, géométriques et constitutionnels. Le coefficient de corrélation (R^2) a été de 0,97 pour cette base de données couvrant 15 systèmes. Le modèle a été validé avec trois autres systèmes.

Chapter 3.6

Modeling the Release of Antioxidants in PLA in Contact with Foods

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Abstract

Antioxidant active packaging is a promising technology for food protection. In this study, the release (diffusion coefficient) of six antioxidants (α -tocopherol, ascorbyl palmitate, BHA, BHT, PG and TBHQ) from a PLA active packaging to food simulants was studied. A model based on the Fick's diffusion equation was used to calculate the diffusion coefficients (D) of antioxidants at 20, 30 and 40°C. Quantitative structure property relationships (QSPR) were developed for calculating diffusion coefficient values based on molecular descriptors. We developed a simple method for estimating diffusion coefficients of 15 systems (PLA/antioxidant/food simulant) of diverse physical and chemical properties such as polarity, ionic force, molar concentration, electronegativity and ionic radii. Our model validated with three systems (at 20 and 40°C). The molecular descriptors were derived from quantum-chemical and geometrical descriptors. The regression coefficient (r^2) for fitting experimental versus predicted release was 0.97 for 15 systems. After the first validation, we used three other systems at 30°C in order to show the efficiency of this model.

1- Introduction

Food packaging is traditionally used to enable marketing of products and to provide passive protection against environmental agents that affect their shelf life (Pereira de Abreu et al., 2011). In recent years, there has been an increased demand for packaging that offers an improved shelf life for fresh, high quality food products (Byun et al., 2010). Active packaging is defined as the packaging that changes the conditions of the packed food to extend shelf life, to improve sensory properties, or to inhibit the growth of pathogenic and spoilage microorganisms (Ahvenainen, 2003)

A novel alternative that is being developed is the design of active antioxidant packaging systems. Various antioxidant agents have been added to polymeric films with the purpose of being delivered to oxygen-sensitive food, improving its chemical stability. Antioxidant packaging is a promising type of controlled release concept in which antioxidants are incorporated into or coated onto food-packaging materials to reduce oxidation of the food. The application of antioxidant-containing packaging films (active packaging) that come into contact with food surfaces may delay lipid oxidation and denaturation of proteins (Torres-Arreola et al., 2007). Some forms of food deterioration, such as the development of off-flavor, color and flavor changes, and nutritional losses, are caused by oxidation (Anklam, 1997; Morales-Aizpurua and Tenuta-Filho, 2005). To prevent oxidation in food products, several synthetic or natural antioxidants have been incorporated into the packaging film structure as an antioxidant packaging (Botterweck et al., 2000; Chen et al., 1992; Hamilton et al., 1997).

Biodegradable materials present a number of promising properties in a number of applications, including packaging, automotive and biomedical sectors. Specifically, thermoplastic biodegradable polymers, such as poly (lactic acid) (PLA), polyhydroxyalkanoates (PHA) and polycaprolactones (PCL), exhibit an excellent equilibrium of properties, apart from being processable using conventional plastics machinery and, for the case of the first two, they arise from renewable resources. PLA is a thermoplastic biopolyester produced from L-lactic acid, which typically comes from the fermentation of corn starch. Currently, PLA is being commercialized as a food packaging polymer in cups, containers and films for short shelf-life products (Auras et al., 2006).

Migration is a mass transfer process by which low molecular mass substances initially present in the package are released into the contained product. Release tests are often analytically complex, tedious and time consuming, but the best approach is to perform release test with real food matrices (Garde et al., 2001; Silva et al., 2007). The migration process has been described by a great number of authors, as Crank (1975), Garde et al. (2001), and Hamdani et al. (1997). Two parameters are important: the diffusion coefficient (D) which measures the rate at which the diffusion process occurs, and the partition coefficient ($K_{p,f}$), defined as the ratio of migrant concentration in the polymeric material (C_p) and its concentration in the food system (C_f) at equilibrium (Tehrany and Desobry, 2004).

Structure/property relationships widely reported in recent years. They are unquestionably of great importance in modern chemistry, biochemistry, physical chemistry properties, medicinal chemistry, biological activities and for food safety. A major goal of quantitative structure property relationship (QSPR) studies is to find a mathematical relationship between the property under investigation (e.g. Pka, partition coefficient, etc.), and one or more descriptive parameters (descriptors) related to molecule structure (Katritzky et al., 1995; Katritzky et al., 1997; Murugan et al., 1994).

The aim of this study was to determine diffusion of antioxidants from PLA packaging to food simulants by an experimental analysis. We have developed a mathematical model for predicting the diffusion of antioxidants in food simulant/packaging active system based on QSPR. When molecules are new or when the experimental determination is rather difficult or subject to large uncertainties, theoretical evaluation method has to be proposed.

2- Materials and methods

Experimental values of diffusion coefficients of antioxidants in food simulant/polymer systems were determined in our laboratory at 20, 30 and 40 °C. The measurements were made on a HPLC (Shimadzu, Japan). HPLC measurements were equipped with a quaternary pump (LC-20AD), an auto-injector (SIL-20AC HT), a UV-Vis photodiode array detector (SPD-M20A), a column oven (CTO-20AC) and Labsolution data software.

The molecular descriptors for each compound were obtained from database of the Molecular Modeling Pro version 5, 2002 (ChemSW Software Inc., USA). All programs and calculations were carried out with MATLAB R2009a Software (The MathWorks Inc., USA). Our program then identified the parameters initially based on a classical second order form.

The model was developed for 15 systems with diffusion coefficients between 1.49×10^{-9} and $8 \times 10^{-12} \text{ cm}^2 \text{ s}^{-1}$.

The general form of the equation was as follows:

$$Eq.1 \quad \mathbf{K} = \mathbf{a}_0 + \sum_i^N x_i \mathbf{a}_i + \sum_{i=1}^{N-1} \sum_{j=i+1}^N x_i x_j \mathbf{b}_{ij} + \sum_{i=1}^N x_i^2 \mathbf{c}_i$$

where a_0, a_i, b_{ij}, c_i are constant, x_i is the i^{th} structural descriptor considered in this model.

Measurement variance (s_y^2) was estimated using the n residues between experimental and modeled D (n "experimental" data were used to fit the model). The variance-covariance matrix of parameters was calculated from Fisher matrix and from the estimated measurement variance (s_y^2). The variance of parameters σ_A^2 thus lay on the diagonal of the resulting matrix.

$$Y = AX$$

where y is the experimental value of D , X is matrix of physical and chemical descriptors of our model and A is the coefficient.

$$\hat{A} = (X^T X)^{-1} X_y^T$$

$$\sigma_A^2 = (X^T X)^{-1} \sigma_y^2$$

$$s_A^2 = (X^T X)^{-1} s_y^2$$

The confidence interval for each parameter is thus given by:

$$A \pm t_{\alpha \delta} \sigma_A \quad (t_{\alpha \delta} \sigma_A = \Delta A) \quad \text{with } \delta = n - N \text{ and } \alpha = 0.95$$

where A : coefficient vector; t : student number; α : confidence level; δ : degree of freedom and ΔA : width of confidence interval.

In this model, we used four parameters: polarity of food, volume, total energy and binding energy of antioxidant. The model was adapted for all diffusion coefficients.

3- Results and discussion

Quantitative measurement of the rate at which a diffusion process occurs is usually expressed in terms of diffusivity, expressed in $\text{m}^2 \text{s}^{-1}$. The classical theory used to model the diffusion process is based on Fick's laws (Crank, 1975; Stannet, 1968).

The amount of package components that may migrate from a plastic packaging material into liquid or solid food depends on the chemical and physical properties of food and polymer (Halek, 1988; Shepherd, 1982). Various factors like migrant concentration, molecular weight, solubility, diffusivity, partition coefficient between polymer and food, time, temperature, polymer and food composition, and structures (density, crystallinity, chain branching) are the main controlling factors in migration (Chang et al., 1988; Gilbert et al., 1980).

In this study, the apparent diffusion coefficients (D) of antioxidants were determined from the release versus time data, which was fitted to Fick's second law for an infinite slab in contact with an infinite volume of solvent were calculated by Eq. (2) (Crank, 1975).

$$\text{Eq. 2} \quad \frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp \left[- \frac{(2n+1)^2 \pi^2}{L^2} D t \right]$$

Where M_t/M_∞ is the concentration of antioxidant diffused at time t, divided by the concentration of antioxidant diffused at equilibrium and L is the thickness of the film.

For short times ($M_t/M_\infty < 2/3$), equation 1 can be simplified to equation 2 and D can be determined with equation 3.

$$\text{Eq. 3} \quad M_t/M_\infty = 4 (D t / 4L^2 \pi)^{1/2}$$

Eq. 4
$$D = (S.L/2)^2 \pi$$

Where, S is the slope of a plot representing M_t/M_∞ against $t^{1/2}$.

The goal of this work was to determine which types of descriptors are the most sensitive to D and also to find both the best regression model for the prediction of diffusion coefficient.

Table 1 presents the experimental diffusion coefficients of antioxidants from PLA to food simulants calculated from Eq. (4).

Table 1. Experimental D values (cm^2s^{-1}) obtained from antioxidants release into ethanolic solutions (95%, 50% and 10%).

	95%	50%	10%	95%	50%	10%	95%
	(40°C)	(40°C)	(40°C)	(20°C)	(20°C)	(20°C)	(30°C)
α -tocopherol	1.49×10^{-9}	nd	nd	2.00×10^{-11}	nd	nd	nd
BHA	4.31×10^{-9}	1.12×10^{-10}	2.28×10^{-10}	3.21×10^{-11}	7.40×10^{-12}	2.28×10^{-10}	6.12×10^{-10}
BHT	3.12×10^{-9}	5.84×10^{-11}	nd	1.60×10^{-11}	1.00×10^{-11}	nd	2.54×10^{-10}
PG	3.90×10^{-9}	1.28×10^{-10}	2.78×10^{-10}	6.12×10^{-11}	7.56×10^{-12}	8.16×10^{-11}	1.77×10^{-9}

n.d: not determined.

3-1- Selection of molecular descriptors and coding of system

In order to simplify the descriptor of the food/migrant/polymer system and the corresponding programming, a code for each „level“ was assigned (Table 2). The food/polymer/migrant at three temperatures was thus described by a single integer code I computed according to:

$$I = 1000 \times L_{\text{Food}} + 100 \times L_{\text{Polymer}} + 10 \times L_{\text{Migrant}} + L_{\text{Temperature}}$$

Table 2. Code for each system.

Food simulant	Polymer	Migrant	Temperature (°C)
10% ethanol	PLA	α -tocopherol	20
50% ethanol		Ascorbyl palmitate	40
95% ethanol		BHA	30
		BHT	
		PG	
		TBHQ	

BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene, PG: Propyl gallate, TBHQ: Tert-butylhydroquinone.

As an example for a system 1131: 10% ethanol/ PLA/BHA at 20°C.

We determined molecular descriptors which best suited our model (quantum-chemical and constitutional descriptors). The Hansen polarity delta/sqr (MPa) of 95%, 50% and 10% ethanol obtained from Molecular Modeling Pro are 8.94, 10.82 and 14.40, respectively. Table 3 shows the physico-chemical properties of food, packaging and migrant.

Table 3. Physico-chemical properties of migrant.

	Volume ^{ac}	Total energy ^{bc}	Binding energy ^{bc}
α -tocopherol	281.94	-282.28	-35.39
Ascorbyl palmitate	244.51	-315.11	-28.64
BHA	109.50	-127.44	-13.24
BHT	143.74	-143.76	-17.87
PG	112.13	-172.45	-12.72
TBHQ	99.44	118.77	-12.02

a: cm³/mol; b: atomic units and c: data from Molecular Modeling Pro.

Among six antioxidants, we calculated the diffusion coefficients of 4 antioxidants (α -tocopherol, BHA, BHT, and PG). It was observed that TBHQ was decomposed to TBBQ and other quinone derivatives in all simulants at 40°C and at weaker degree in 95% and 50% ethanol at 20°C. Ascorbyl palmitate was completely degraded during PLA film preparation and did not show any release in none of the simulants.

We studied 15 different systems of food/migrant/packaging and using quantum-chemical and geometric descriptors, calculated the diffusion coefficient values from Eq. 5. In this model, we calculated the natural logarithm of diffusion coefficient because of several decades of experimental values.

Eq. 5

$$\ln(D) = a_0 + a_1 \cdot X_1 + a_2 \cdot X_2 + a_3 \cdot X_3 + a_4 \cdot X_4 + a_5 \cdot X_1 X_2 + a_6 \cdot X_1 X_4 + a_7 \cdot X_2 X_4 + a_8 \cdot X_1^2$$

Our model was based on four molecular descriptors: the polarity of food simulant (x_1), the volume of migrant (x_2), the total energy (x_3), and the binding energy of migrant (x_4).

Common sense and chemical intuition suggest that the higher the temperature, the faster a given chemical reaction will proceed. Quantitatively this relationship between the rate of reaction proceeds and its temperature is determined by the Arrhenius Equation. At higher temperatures, the probability that two molecules will collide is higher. This higher collision rate results in a higher kinetic energy, which has an effect on the activation energy of the reaction. The activation energy is the amount of energy required to ensure that a reaction happens.

In order to determine the temperature dependence of the diffusion coefficient, the well-known Arrhenius equation (Eq. 6) was used (Chatwin, 1996):

Eq. 6
$$D = D_0 \exp\left(\frac{-E_a}{RT}\right)$$

Where D_0 is a pre-exponential factor; E_a is the activation energy; R is the ideal gas constant and T is the absolute temperature.

The parameters D_0 and E_a can be obtained by curve fitting of the experimental data (Helmroth et al, 2002). In this study, we used the Arrhenius Equation taking into account some coupling

between temperature, energy and polarity. Two temperatures (20 and 40°C) were used for developed model and we validated this model with a third temperature (30°C).

The developed model was shown in Eq. 7.

Eq. 7

$$\ln(D) = a_0 + a_1x_1 + a_2 \ln(x_2) + a_3 \left(-\frac{x_3}{RT} \right) + a_4 \left(-\frac{x_4}{RT} \right) + a_5x_1 \ln(x_2) + a_6x_1 \left(-\frac{x_4}{RT} \right) + a_7x_2 \left(-\frac{x_4}{RT} \right) + a_8x_1^2$$

We validated our model with three different systems, which were not included in the identification data set. Correlation coefficient value (r^2) for Eq. 7 and model validation was 0.97. The coefficients of model were showed in Table 4.

Table 4. Coefficients of Eq 7.

a_0	-334.14
a_1	19.69
a_2	102.29
a_3	-105.23
a_4	-41062.61
a_5	-7.67
a_6	1322.61
a_7	4247.77
a_8	0.40

3-2- Calculated and experimental D values

Fig 1 presents the relationship between Ln (D) calculated by Eq. 7 and the experimental values calculated from Eq. 4. Three validation values were specified by \circ and identification values were determined with \times . The goal of these studies is to find both the best regression model for the prediction of Ln (D) and the types of descriptors most sensitive to Ln (D). We

validated our model by three other systems (95% ethanol/PLA/antioxidants) storage at 30°C presented with \square . The antioxidants used in this part were BHA, BHT and PG. The result showed that the developed model is valid for the temperature between 20 and 40°C (Fig 1).

The fact that the relationship between diffusion and temperature is well described in the present study by the Arrhenius equation, suggests that the effect of temperature is thermodynamic in nature, regulated essentially by the proportion of energy provided to the activation energy (Daniels and Alberty, 1972).

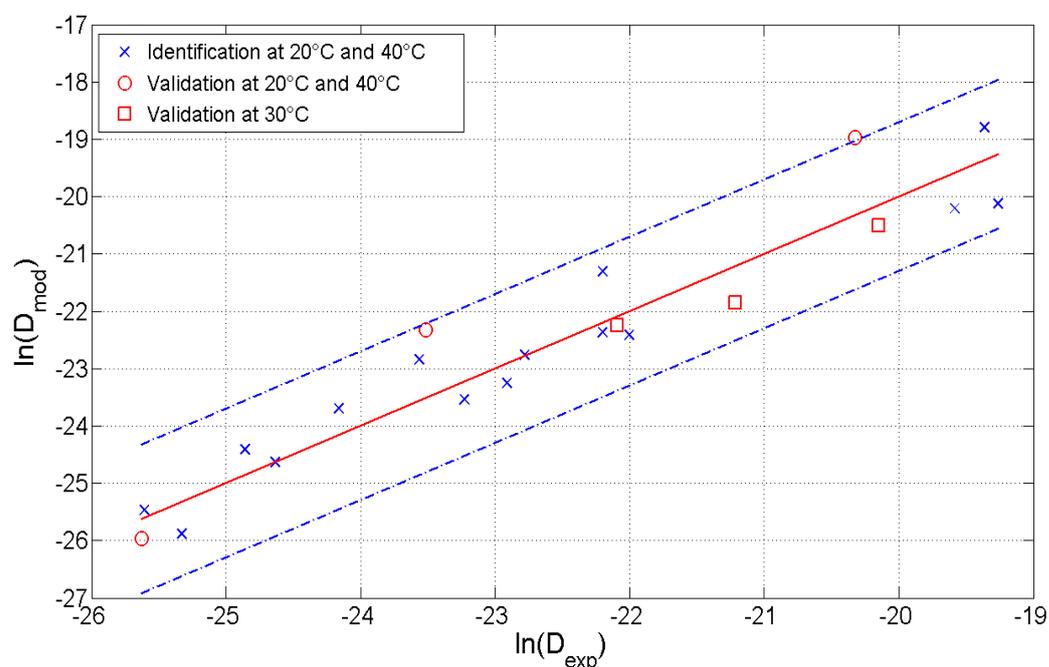


Fig. 1. Correlation between experimental and calculated $\ln(D)$ from Eq 7.

Although, combination of two types descriptors (geometrical and Quantum-chemical descriptors) gave a good result.

Geometric descriptors represent the three-dimensional aspects of the structures such as molecular size and molecular shape. Three-dimensional molecular shape is often an important determinant of the physico-chemical properties or biological activities of chemical compounds. It included such descriptors as moments of inertia, shadow areas, solvent

accessible surface area, gravitational index and molecular volume (Ghose et al, 1988; Rohrbaugh and Jurs, 1987).

Quantum-chemical descriptors group all properties of a molecule related to its electron distribution and behavior and it is therefore not surprising that fundamental properties obtained through quantum chemical calculations have been used in the quantitative description of biological activity (Dearden, 1990). These descriptors are a rapidly developing class of molecular descriptors. Quantum-chemical calculations provided varied information about chemical structure including geometric and electrostatic data. They include the energy of the highest occupied and lowest unoccupied molecular orbital electron densities, Mulliken population charge distribution, and dipole moments (Buydens et al., 1983; Kikuchi, 1987; Sklenar and Jäger, 1979).

A semi-empirical quantum chemistry program used in our research to calculate total energy and binding energy of a molecule.

Volume of antioxidant can be important because the size of the molecule had a great impact on amounts absorbed, larger molecules being absorbed to a larger extent than the smaller ones and highly branched molecules were absorbed to a greater extent than linear molecule.

A semi-empirical quantum chemistry program (CNDO) that we have used in our model to calculate the total energy and binding energy of a molecule. This program is known as semi-empirical because it is based on a mixture of first principles of chemistry and physics with experimental results that is used to determine the Hamiltonian. Programs doing quantum chemistry based only on first principles are known as „ab initio“ calculations. CNDO uses two main approximations that deviate from ab initio: (a) a core approximation and (b) the zero-differential overlap approximation.

Most ab initio calculations have been based on the orbital approximation. In general, this method provides better results the larger basis set (i.e. number of atomic orbitals) employed, although according to the variational principle this is strictly valid for the total electron energy of the molecule. The ab initio model Hamiltonian provides a complete representation of all non relativistic interactions between the nuclei and electrons in a molecule.

The total energy is the sum of the total one-center and two-center terms. The one-center energy terms include electron-electron repulsion and electron-nuclear attraction.

The two-center energy terms include resonance energy, exchange energy, electron-electron repulsion, electron-nuclear attraction, and nuclear-nuclear repulsion. Binding energy is the difference between the total energy of the equilibrium molecular geometry and the sum of the atomic energies of the constituent atoms

The distribution of the electrons within a covalent bond determines its polarity. When electrons are shared equally among the atoms, the bond has a non-polar character, but when electrons are shared unequally, the bond has a polar character (Atkins, 1994; Karplus and Porter, 1970). Polarity of the molecule depends on the symmetry of the various covalent bonds which it contains.

4- Conclusion:

In this study, quantitative structure-property relationships (QSPR) were developed for the calculation of $\ln(D)$ values based on molecular descriptors. We studied 15 different systems of food/migrant/packaging by using quantum-chemical and geometric descriptors. This model was based on four molecular descriptors: polarity of food simulant, volume, total energy and energy binding of antioxidants. The model was validated with three different systems. The theoretical values of $\ln(D)$ showed that there is a good correlation between the physico-chemical and structure of molecule. In a near future QSPR models will be more and more developed to allow prediction of diffusion coefficient and then estimate chemical food safety. This model will be expanded in real food/packaging system with addition some properties of food and packaging on this present model.

5- References

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IV . Conclusion et perspectives

Le PLA offre de grandes possibilités dans une large gamme d'applications de produits d'emballage alimentaire et rivalise avec le polystyrène. L'objectif principal de cette étude était de développer un emballage biodégradable actif pour la libération d'antioxydants afin de contrôler en permanence le processus d'oxydation dans les aliments. Le PLA en tant que polymère biodégradable industriellement produit et les antioxydants autorisés tels que l'alpha-tocophérol, l'ascorbyle palmitate, le BHA, le BHT, le PG et le TBHQ ont été choisis en raison de leurs applications dans les industries alimentaires et pharmaceutiques. Les objectifs de cette thèse étaient d'approfondir les connaissances sur le mode d'incorporation des antioxydants dans les matrices de PLA, de caractériser ces systèmes d'un point de vue multi-échelles, d'identifier les phénomènes de migration des antioxydants dans les aliments simulants et enfin, de développer un modèle mathématique afin de pouvoir prévoir les cinétiques de libération contrôlée.

Dans la première partie de ce travail, les films de PLA contenant des antioxydants (naturels et synthétiques) ont été fabriqués afin de les étudier pour les utiliser en tant qu'emballage alimentaire. Il a été démontré que l'ajout de 1% d'antioxydants synthétiques dans les films de PLA fabriqués par la méthode de « solvant-cast » a pour effet de réduire certaines caractéristiques physiques et mécaniques, telles que la cristallinité (26%) des films de PLA quel que soit l'antioxydant. Le module d'Young et la résistance à la traction des films de PLA ont été légèrement affectés par le BHA, le PG et le TBHQ. La perméabilité à la vapeur d'eau du PLA n'a pas été modifiée par l'ajout d'antioxydants, mais la perméabilité à l'oxygène du PLA a été modifiée uniquement pour le BHT. Les résultats d'XPS ont montré que les deux faces des films de PLA contenant 1% d'antioxydant n'avaient pas exactement la même composition et ce qui suggère que les antioxydants n'étaient pas répartis uniformément dans l'épaisseur des films en raison de la décantation et de la précipitation partielle des antioxydants au cours du séchage de la solution de polymère. La microstructure des films de PLA a été significativement modifiée par l'addition des antioxydants (rugosité R_a et R_q). Les antioxydants synthétiques n'ont pas eu d'action significative de modification des propriétés du film de PLA et leurs utilisations pour la création d'un emballage actif à base de PLA sont techniquement sûres.

La cristallisation de l'ascorbyle palmitate (AP) à la surface des films de PLA les a rendus opaques ce qui va à l'encontre des souhaits des consommateurs. De plus, la perte de 35% d'AP lors de la préparation des films est élevée et risque d'être encore accrue lors de la fabrication

industrielle de polymères impliquant des températures et pressions élevées. L'alphatocophérol (AT) est intégré différemment à la surface des films de PLA. En effet, l'énergie de surface du verso des films est plus élevée que le recto ; ce changement a également été observé dans les composantes polaires et de dispersion. Les résultats XPS ont également attesté de l'influence de l'antioxydant AT sur la composition chimique des films de PLA au verso. L'AFM a montré que les films minces de PLA-AT ont une rugosité comparable aux films de PLA pur, alors que l'ajout d'AP aux films de PLA a augmenté les paramètres de rugosité R_a et R_q . Nous avons observé une influence de l'antioxydant AT sur les propriétés thermiques des films de PLA-AT au niveau de leur température de cristallisation par rapport au film de PLA. Les antioxydants AP et AT réduisent légèrement la cristallinité de films qui par conséquent influe sur les propriétés mécaniques des polymères. Selon nos résultats, les antioxydants n'ont pas d'effet sur la perméabilité à la vapeur d'eau des films PLA malgré la modification de la structure cristalline de surface provoquée par l'AP. En considérant l'effet négatif de l'AP sur la transparence des films de PLA, la forte perte d'antioxydant pendant la préparation des films de polymère et le faible apport au niveau des propriétés mécaniques, nous pouvons en conclure que l'AP n'est pas un antioxydant approprié pour l'incorporation dans des emballages actifs à base de PLA. Au vu de ces considérations, il est préconisé de continuer à utiliser directement cet antioxydant dans les formulations alimentaires. D'autre part, bien que l'AT n'ait pas non plus d'effet notable sur les propriétés des films de PLA, nous considérons que cet antioxydant reste approprié et sûr à la création d'emballages actifs de PLA en raison de sa bonne tenue lors de son incorporation dans la matrice de polymère.

Pour une meilleure compréhension du mode d'intégration des antioxydants dans les matrices de PLA, une concentration plus élevée d'antioxydants a été incorporée au PLA. Les propriétés macro, micro et nano des films de PLA-antioxydants ont été étudiées. Différentes couches de films de PLA-antioxydants de la couche superficielle aux couches profondes ont été étudiés en utilisant la diffraction des rayons X, la spectroscopie Raman, la spectroscopie FTIR et l'AFM et la MET. La température de cristallisation du PLA-antioxydant augmente, sauf pour le PLA-AT. Aucun des antioxydants n'a eu d'effet sur les films de PLA excepté une faible augmentation de l'élongation à la rupture pour les films de PLA-AT. Au contraire, en ajoutant de l'AT ou de l'AP, la composition des films de PLA a changé sur les deux faces pour l'AT et uniquement sur le recto pour l'AP. La raison majeure pour une modification des propriétés du PLA-AP côté recto est la cristallisation d'AP sur la surface des films ayant pour résultat une

augmentation de l'opacité du film. Tous les antioxydants ont augmenté l'intensité des spectres Raman à 1774, 1768, 1751 et 1172 cm^{-1} ce qui signifie une augmentation des phases cristallines. De plus, quelques nouvelles bandes pour le PLA-PG sont apparues dans la région de 1700-1600 cm^{-1} . L'XPS a montré une modification importante de la surface des films due aux cristaux d'AP. Les images de MET ont montré qu'à part les films de PLA-AP, les films de PLA-antioxydants ont deux structures différentes sur les faces recto et verso des films à cause de la précipitation partielle des antioxydants au cours de l'évaporation du solvant. L'AFM a montré que les films minces de PLA-AT ont une rugosité très similaire aux films minces de PLA pur. Les films de PLA-BHT et le PLA-BHA ont présenté une microstructure qui peut être associée à des microfibrilles. L'ajout de 5% de TBHQ induit à la surface une microstructure fragmentée de microfibrilles et des particules de forme allongée. En ajoutant 5% de PG, la microstructure en surface varie considérablement par rapport aux autres surfaces. Globalement, on peut conclure qu'à part l'AP, les antioxydants sont incorporés dans la phase amorphe de la matrice de PLA et n'interagissent pas chimiquement avec les groupes fonctionnels du PLA. Nous observons une cristallisation de l'AP à la surface de PLA et une modification de l'opacité de ce film.

La deuxième partie de ce projet traitait du comportement de migration des antioxydants des films de PLA vers les aliments simulants à 20°C et 40°C. L'AP a été complètement dégradée lors de la préparation des films de PLA et n'a montré aucune migration dans aucun des aliments simulés. La libération des antioxydants a suivi un comportement de diffusion du type Fickien. En général, les principaux facteurs affectant la libération des antioxydants dans des simulants éthyliques incluent: la polarité du simulant, la solubilité de l'antioxydant, l'épaisseur du film, la température, l'agitation, le poids moléculaire et la dégradation du polymère. La polarité élevée de l'éthanol 10% a agi comme une barrière et a empêché la libération des molécules d'AT, BHA, BHT et TBHQ à 20°C, mais l'augmentation de la mobilité moléculaire des antioxydants BHA et TBHQ par l'élévation de la température à 40°C a permis de surmonter cet obstacle. Le TBHQ a été décomposé en TBBQ et dérivés quinone dans tous les simulants à 40°C et dans une moindre mesure dans l'éthanol 95% et 50% à 20°C. Des composés néoformés ont également été détectés pour l'AT dans l'éthanol 50% à 40°C avec des caractéristiques plus hydrophobes. Les différentes vitesses de libération des antioxydants à partir du PLA et leur concentration finale migrée vers l'aliment simulant sont décrites par les coefficients de diffusion (D) et de partage (K). Ils montrent la possibilité

d'utiliser le PLA comme support d'antioxydants afin de réguler en permanence les réactions d'oxydation des aliments.

La libération de BHA, BHT, PG et TBHQ des films de PLA extrudés vers les simulants à 20°C et à 40°C a été étudiée en appliquant les mêmes méthodes analytiques que pour les films obtenus par casting. Le taux d'antioxydants libérés à 40°C était du même ordre de grandeur que les films « solvant-cast ». A 20°C, TBHQ et PG ont montré un plus haut taux et une libération plus rapide dans l'éthanol 95% et 50%, respectivement pour les films de PLA solvant-cast et PLA extrudé. Aucune libération d'antioxydants n'a été observée dans l'éthanol 10% pour le PLA extrudé excepté pour le PG qui a une légère migration dans l'éthanol 10% pour les films de PLA solvant-cast. Le TBHQ s'est décomposé en TBBQ pendant le procédé d'extrusion et de tests de migration quelles que soient les conditions. Le BHT avait le plus faible taux de libération dans tous les cas et n'avait pas migré au contact de l'éthanol 10%. Comme prévu pour les migrants apolaires, le coefficient de diffusion apparents des antioxydants a diminué avec l'augmentation de la polarité du simulant. En général, les facteurs principaux affectant la migration des antioxydants synthétiques du PLA extrudé dans les simulants éthyliques comprennent: les propriétés des antioxydants notamment leur solubilité dans le simulant, le Log P, la polarité, le poids et le volume moléculaires, la polarité du simulant, l'épaisseur du film, la température, l'agitation et la dégradation du polymère. Les coefficients de diffusion des antioxydants pour les PLA solvant-cast et extrudé ne sont pas très différents et, dans certains cas, le coefficient de diffusion des antioxydants du PLA solvant-cast était plus faible que celui du PLA extrudé ce qui peut être attribuée à une distribution plus uniforme des antioxydants dans le PLA extrudé. Les emballages actifs de PLA-antioxydants pour divers produits alimentaires peuvent être pratiquement réalisés en utilisant les résultats de cette étude et en ajoutant des données supplémentaires pour d'autres antioxydants impliquant leurs effets synergiques. La libération rapide et totale des antioxydants dans l'éthanol 95% et aussi leur libération lente et faible dans l'éthanol 10% a montré la pertinence d'un emballage actif de PLA-antioxydants pour les huiles, les aliments gras et certains produits laitiers qui sont réellement sensibles à l'oxydation.

Enfin, un modèle mathématique des coefficients de diffusion des antioxydants du PLA solvant-cast vers des aliments simulants a été développé en utilisant un programme sur Matlab développé au laboratoire. Les relations quantitatives structure-propriété (QSPR) ont été développées pour le calcul des valeurs de $\ln(D)$ fondées sur des descripteurs moléculaires.

Nous avons étudié 15 systèmes différents d'aliments/migrants/emballages en utilisant les descripteurs quantiques-chimiques et géométriques. Ce modèle est fondé sur quatre descripteurs : la polarité des aliments simulés, le volume moléculaire, l'énergie totale et l'énergie de liaison des antioxydants. Les valeurs théoriques de $\ln(D)$ ont montré qu'il existe une bonne corrélation entre les caractéristiques physico-chimiques et la structure de la molécule. Ce modèle sera étendu à des systèmes d'aliments réels/emballage avec l'ajout de propriétés des aliments et de l'emballage.

Perspectives

L'oxydation des lipides insaturés est une cause majeure de détérioration de la qualité des aliments, donnant lieu à l'apparition de mauvais goût et à une perte de valeur nutritionnelle des produits alimentaires. Les préoccupations sociétales concernant la santé et les antioxydants utilisés dans les aliments et les médicaments sont en augmentation permanente. Afin de minimiser la quantité des antioxydants directement ajoutés dans les aliments, les emballages actifs contenant des antioxydants sont des solutions prometteuses. La pertinence de l'utilisation du PLA, polymère industriel biocompostable, pour créer un emballage antioxydants actifs a été étudiée dans ce projet. Pour envisager une réelle application industrielle certains aspects doivent encore être étudiés :

- L'incorporation d'autres additifs alimentaires autorisés dans le PLA pourrait être intéressante; leurs migrations probables vers des produits alimentaires ne menacera pas la sécurité des produits emballés.
- La libération rapide et totale des antioxydants dans l'éthanol 95% à 40°C montre la nécessité d'études complémentaires pour maîtriser leur libération progressive pour assurer un contrôle continu et durable de l'oxydation.
- Comme nous avons étudié la libération des antioxydants dans les aliments simulés, des études complémentaires à l'aide d'aliments réels au contact de films de PLA-antioxydants sont nécessaires pour réaliser ce projet en conditions réelles. Les aliments solides ou pâteux présenteront des comportements diffusionnels différents intéressants à étudier.
- Pendant l'étude de la libération, la décomposition de l'alpha-tocophérol et du TBHQ en composés néoformés sont survenues. Nous n'avons été en mesure que de détecter le TBBQ issu de l'oxydation du TBHQ. Des études supplémentaires sont nécessaires pour enquêter sur la nature des autres composés et sur leurs mécanismes de formation.

D'autres biomatériaux peuvent également être envisagés :

- Des mélanges de PLA avec d'autres biopolymères tels que l'hydroxypropylméthylcellulose (HPMC), l'acétate de cellulose, le PGA, ou le chitosan peuvent être développés pour fournir un emballage actif avec une libération contrôlée des antioxydants.

- Une extension du modèle développé à d'autres antioxydants ou agents actifs ainsi qu'à d'autres polymères semblables au PLA peuvent améliorer les applications pour assurer une meilleure sécurité des produits alimentaires.

PLA offers great promise in a wide range of applications and competes with polystyrene for many food packaging. The principal objective of this study was to develop a biodegradable active packaging for antioxidant release to continually control the oxidation process in foods. PLA as the best well known biodegradable polymer and food permitted antioxidants such as alpha-tocopherol, ascorbyl palmitate, BHA, BHT, PG and TBHQ were chosen because of their common use in food and pharmaceutical industries. Knowledge about the antioxidants mode of incorporation in PLA matrix, the multi-scale characterization of functionalized film, the antioxidants release behaviors in food simulants and finally developing a corresponding model is perquisite to reach the objective of the present thesis work.

In the first part of this project, PLA films containing antioxidants (natural and SPA) were prepared using chloroform and their suitability to fulfill basic packaging functions was investigated. It was demonstrated that addition of 1% of SPAs to PLA films by casting method results in changing some physical and mechanical characteristics including 26% reduction in PLA crystallinity by all SPAs. The Young's modulus and tensile strength of PLA film were slightly affected by BHA, PG and TBHQ. Water vapor permeability (WVP) of PLA was not changed by 1% addition of SPAs and the oxygen permeability of PLA film was only influenced by BHT. XPS results showed that two different faces of PLA films containing 1% of SPAs were different and this suggested that SPAs were not uniformly distributed through film thickness because of partial antioxidant precipitation during polymer solution drying. PLA film microstructures were significantly changed by addition 1% of SPAs; in term of roughness parameters (Ra and Rq). In one hand, SPAs did not significantly modify PLA film properties and in the other hand their uses for creating active PLA packaging was technically safe.

Ascorbyl palmitate (AP) crystallization at PLA surface changed transparent PLA to an opaque film which was not desirable in term of consumers' acceptability. Additionally 35% loss of AP during film preparation was high and obviously this loss will be intensified by industrial polymer fabrication implying elevated temperatures and pressures. Alpha-tocopherol (AT) differently incorporated in PLA surfaces because the verso face of PLA-AT had higher surface energy than the recto one, this change was also observed in both polar and dispersion components. XPS results also proved that AT influenced chemical composition of PLA film at verso side. AFM showed PLA-AT thin films had a near similar roughness comparing to PLA, while by adding the AP to PLA film, the roughness parameters including Ra and Rq

were increased. The noticeable influence of AT on PLA thermal properties was found on PLA crystallization temperature. AP and AT slightly reduced PLA crystallinity which consequently affected polymer mechanical properties. The antioxidants influenced the water vapor permeability of PLA film in spite of crystalline surface structure caused by AP. Considering the negative effect of AP on PLA transparency, having no significant modification influence on PLA properties and finally its high loss during polymer preparation, it can be concluded that AP is not a suitable antioxidant for PLA-active packaging and suggested to be used directly in food formulations. In one hand, AT also had no a noticeable modification effect on PLA film properties but in the other hand its uses for creating PLA as a suitable delivering material is technically safe.

For a better understanding of antioxidants mode of incorporation in PLA matrix, a higher concentration of antioxidants was impregnated to PLA using chloroform. Macro, micro and nano properties of PLA-antioxidant films were investigated. Diverse thickness layers of PLA-antioxidant films from superficial to profound layers were studied using X-ray photoelectron, Raman spectroscopy, FTIR spectroscopy, AFM and TEM. PLA crystallization temperature increased by adding antioxidants, except for PLA-AT. None of antioxidants modified PLA regardless a little increase of elongation at break made by AT. On contrary, by adding the AT and AP, composition of PLA changed in both recto and verso sides for AT and just in the recto side for AP. The major reason for PLA-AP recto side difference is AP crystallization on film surface resulting in an opaque film. All antioxidants increased the Raman intensity at 1774, 1768, 1751 and 1172 cm^{-1} meaning the increase in both crystalline and amorphous phases. Some new bands for PLA-PG appeared in the region of 1700-1600 cm^{-1} . XPS showed a large modification of PLA surface made by AP crystals. TEM images showed that, except PLA-AP, PLA-antioxidant film had two different structures at recto and verso sides due to partial precipitation of antioxidants during solvent evaporation. AFM showed that PLA-AT thin films had a near similar roughness with PLA thin films. By adding the AP in PLA film, the roughness parameters, R_a and R_q , increased. PLA-BHT and PLA-BHA presented a homogeneous microstructure which can be associated with well-ordering microfibrils. Adding 5% of TBHQ induced fragmented microfibrils surface microstructure and grain-like particles appeared with some remained rod shaped. By adding 5% of PG, the surface microstructure varied significantly compared to other surfaces. Globally, it can be concluded that except AP, other antioxidants incorporated in PLA matrix inside the amorphous phase and had no real

chemical reaction with PLA functional groups. AP did not fully participate in PLA matrix and crystallized in PLA surface changing PLA transparency.

Second part of this project comprised the antioxidants release behaviors from solvent-cast PLA in food simulants at 20°C and 40°C. AP was completely degraded during PLA film preparation and did not show any release in none of the simulants. In general, main factors affecting antioxidant release from PLA into ethanolic simulants include: simulant polarity, antioxidant solubility in simulant, film thickness, temperature, stirring, antioxidant molecular weight and polymer degradation. The high polarity of 10% ethanol acted as a barrier and prevented the release of AT, BHA, BHT and TBHQ at 20°C, but increased molecular mobility of BHA and TBHQ provided at 40°C overcame this barrier. TBHQ was decomposed to TBBQ and other quinon derivatives in all simulants at 40°C and at weaker degree in 95% and 50% ethanol at 20°C. Some neoformed compounds also was detected from AT in 50% ethanol at 40°C with more hydrophobic characteristics. The various velocities of antioxidants release from PLA and their final migrated concentration to simulants were described by diffusion (D) and partition (K) coefficients. PLA could become a good carrier for antioxidants to continually control food oxidation reactions.

Consequently, the release of BHA, BHT, PG and TBHQ from extruded PLA films into simulants at 20°C and 40°C was studied applying the same analytical methods. Antioxidants release showed a Fick behavior. The rate of antioxidants release at 40°C had the similar order as solvent cast films. At 20°C, TBHQ and PG had the highest and fastest rate of release in 95% and 50% from solvent-cast and extruded PLA, respectively. No antioxidant release was observed in 10% ethanol for extruded PLA while PG slightly released from solvent-cast film. TBHQ decomposed to TBBQ both during extrusion process and release tests under all conditions. BHT had the lowest rate of release in all cases and completely retained in PLA in contact with 10% ethanol. As expected for apolar migrants, the D coefficient of antioxidants decreased by increased simulant polarity. In general, main factors affecting SPAs release from extruded PLA into ethanolic simulants include: antioxidant properties such as solubility, Log P, polarity, molecular weight and molecular volume, simulant polarity, film thickness, temperature, stirring, and polymer degradation. The D coefficients of antioxidants for solvent-cast and extruded PLA were not so different and in some case apparent D coefficient of antioxidant in solvent-cast PLA was smaller than one in extruded PLA which can be attributed to tortuosity change due to more uniform distribution of antioxidants in extruded

PLA. PLA-antioxidant active packaging for various foodstuffs can be practically achieved by using the results of this study and also the supplementary data for other antioxidants involving their synergetic effects. Rapid and total release of antioxidants into 95% ethanol and also their small and slow release into 10% ethanol showed the suitability of PLA-antioxidant active packaging for oils, oily foods and some milk products which are actually in danger of oxidation.

Finally, a mathematical model using D coefficients of antioxidant in solvent-cast PLA was developed applying Matlab software. The quantitative structure-property relationships (QSPR) were developed for the calculation of $\ln(D)$ values based on molecular descriptors. We studied 15 different systems of food/migrant/packaging by using quantum-chemical and geometric descriptors. This model was based on four molecular descriptors: polarity of food simulant, volume, total energy and energy binding of antioxidants. The theoretical values of $\ln(D)$ showed that there is a good correlation between the physico-chemical and structure of molecule. This model will be expanded in real food/packaging system with addition of food and packaging properties.

Perspectives

Oxidation of unsaturated lipids is a major cause of food quality deterioration by giving rise to the development of off-flavor compounds and loss of nutritional value of food products. Health concerns about synthetic antioxidants used in foods and drugs are increasing every day. To minimize the amount of directly added antioxidants, antioxidant active packaging is a promising tool. The suitability of PLA, the best industrial biodegradable polymer, for being an antioxidant active packaging was studied this project. The following perspectives appears now to envisage active antioxidant PLA use in the industry :

- Incorporation of other food additives into PLA with purpose of PLA modification might be interesting.
- The fast and total release of antioxidants into 95% ethanol at 40°C shows the requirement of further studies to decelerate their release for ensuring a continual control of oxidation.
- As we studied the release of antioxidants into food simulants, complementary studies using real foods in contact with PLA-antioxidant films are needed to fulfill this project in real conditions. It is probable that solid or viscous models will offer interesting and very different behavior for antioxidant release.
- During release study, the decomposition of alpha-tocopherol and TBHQ into neoformed compounds occurred. We were able to detect TBBQ from TBHQ oxidation and further studies are needed to investigate the nature of these compounds and their formation mechanisms.

Moreover of biomaterials can be envisaged:

- The blends of PLA with other biopolymers such hydroxypropyl methylcellulose (HPMC), cellulose acetate, PLGA, chitosan, etc... to provide a controlled release antioxidant active packaging can be developed.
- The investigation of developed model for the migration of other antioxidants or active agents from polymer similar to PLA can improve its applications for ensuring the safety of food products.

AUTORISATION DE SOUTENANCE DE THESE
DU DOCTORAT DE L'INSTITUT NATIONAL
POLYTECHNIQUE DE LORRAINE

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VU LES RAPPORTS ETABLIS PAR :

Monsieur Frédéric DEBEAUFORT, Professeur, Université de Bourgogne, Dijon

Monsieur Gilles TRYSTRAM, Professeur, ENSIA, Massy

Le Président de l'Institut National Polytechnique de Lorraine, autorise :

Monsieur JAMSHIDIAN Majid

à soutenir devant un jury de l'INSTITUT NATIONAL POLYTECHNIQUE DE LORRAINE,
une thèse intitulée :

**"Inclusion et libération de molécules antioxydants dans un emballage à base d'acide
poly lactique en contact alimentaire. "**

en vue de l'obtention du titre de :

DOCTEUR DE L'INSTITUT NATIONAL POLYTECHNIQUE DE LORRAINE

Intitulé du doctorat : « **Procédés Biotechnologiques et Alimentaires** »

Fait à Vandoeuvre, le 5 Décembre 2011

Le Président de l'I.N.P.L.,

F. LAURENT



Inclusion et Libération de Molécules Antioxydantes dans un Emballage à Base d'Acide Poly Lactique en Contact Alimentaire

Résumé

Les emballages actifs permettent d'étendre la durée de conservation des aliments, réduisent l'utilisation d'additifs et de conservateurs dans les préparations alimentaires, préservent mieux les saveurs et la qualité des aliments. La libération contrôlée des antioxydants à partir d'emballages alimentaires étend la stabilité des produits (oxydation des lipides réduite) par enrichissement continu en antioxydants alimentaires en surface de l'aliment. L'objectif général du présent travail était d'étudier l'applicabilité de l'acide poly lactique (PLA, polymère biodégradable fabriqué industriellement) comme l'emballage actif. Pour cela, nous avons choisi plusieurs antioxydants synthétiques ou naturels, comme l'alpha-tocophérol, le palmitate d'ascorbyle, le BHA, le BHT, le gallate de propyle et le TBHQ pour produire les emballages antioxydants. En premier lieu, le mode de d'inclusion de ces antioxydants dans la matrice de PLA et leurs effets sur diverses propriétés structurale, thermique, mécanique et barrière du PLA ont été étudiés. L'étude la libération des antioxydants a été réalisée à partir de films de PLA-antioxydants en contact avec trois simulateurs d'aliments (95%, 50%, et 10% d'éthanol) à deux températures (20°C et 40°C). Les résultats ont montré que le PLA a la capacité de contenir et de libérer des antioxydants dans certains produits alimentaires. Enfin, un modèle mathématique basé sur des relations quantitatives structure/propriété (QSPR) a été développé pour prédire la diffusion antioxydants dans les systèmes aliments simulés /emballage actif.

Mots-clés: PLA, antioxydants, caractérisation, emballages actifs, modélisation QSPR.

Inclusion and Release of Antioxidants in Poly Lactic Acid Film in Contact with Foods

Abstract

Active packaging generates longer shelf-life foods, lower usage of additives and preservatives in food formulations, higher protection of flavors and higher food qualities. Antioxidant controlled release from packaging provides longer food stability (reduced lipid oxidation) by continuously liberating antioxidants at food surface. The overall objective of the present work was to study the suitability of Poly Lactic Acid (PLA, biodegradable polymer industrially produced) as active packaging. We chose various synthetic or natural antioxidants including alpha-tocopherol, Ascorbyl palmitate, BHA, BHT, Propyl gallate and TBHQ to produce the antioxidant packaging. Firstly, the mode of incorporation of these antioxidants in PLA matrix and also their potential effects on diverse structural, thermal, mechanical and barrier properties of PLA were investigated. The release study of antioxidants was accomplished from PLA-antioxidants films into three food simulants, i.e. 95%, 50%, and 10% ethanol at two temperatures (20°C and 40°C). The results showed that PLA has the capability for being as a suitable carrier for antioxidant-active packaging for some food products. Finally, a mathematical model based on quantitative structure property relationships (QSPR) was developed to predict antioxidant diffusion in food simulant/ active packaging system.

Keywords: PLA, antioxidants, characterization, active packaging, QSPR modeling.