Active PLA/EVA packages incorporating polyethylene glycol and chitosan as proprietary modifying agents for bread applications

Embalagens ativas de PLA / EVA com incorporação de polietilenoglicol e quitosana como agentes modificadores de propriedade para aplicações em embalagens de pão

Envases activos de PLA / EVA con incorporación de polietilenglicol y quitosano como agentes modificadores de propiedades para aplicaciones de envasado de pan

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Abstract
This work focused on the development of biodegradable active packaging with poly(lactic acid) (PLA), poly(ethylene-co-vinyl acetate) (EVA), polyethylene glycol (PEG) and chitosan (QUI) blends. It investigated thermal and mechanical morphological characteristics of the blends, as the same time, the antifungal activity of the packaging. To assess the antimicrobial activity of the PLA/EVA/PEG/QUI blends, the samples were inserted between slices of bread with no preservative to the evaluation of their shelf life. By comparing between PLA/EVA/PEG, PLA/EVA/PEG/QUI blends and neat PLA was possible to evidence the partial miscibility, decreased glass transition temperature (T_g) by incorporating PEG into the blends, a decrease in flexural strength of 71% and elasticity modulus of 80.4% to PLA/EVA/PEG/2.5QUI blend, as well as an increase in elongation at break of 153% and 392% to impact toughness. A similar behavior was observed to PLA/EVA/20PEG and PLA/EVA/PEG/5.0QUI. The QUI-containing film among the bread slices has also influenced the water activity reduction, and reduced about 35% in the count of molds and yeasts in the slices of bread. Chitosan in mixtures with PLA/EVA/PEG showed potential as a natural antifungal agent in bakery packaging.
Keywords: Poly(lactic acid); Polyethylene glycol; Poly(ethylene-co-vinyl acetate); Chitosan; Antimicrobial; Bread; Packaging applications.

Resumo
Este trabalho teve como foco o desenvolvimento de embalagens ativas biodegradáveis com misturas de poli (ácido lático) (PLA), poli (etileno-co-vinil acetato) (EVA), polietilenoglicol (PEG) e quitosana (QUI). Foram investigadas as características morfológicas térmicas e mecânicas das misturas e, ao mesmo tempo, a atividade antifúngica da embalagem. Para avaliação da atividade antimicrobiana das misturas de PLA/EVA/PEG/QUI, as amostras foram inseridas entre fatias de pão sem conservante para avaliação de seu prazo de validade. Ao comparar entre as misturas poliméricas de PLA/EVA/PEG e PLA/EVA/PEG/QUI e o PLA puro foi possível evidenciar a miscibilidade parcial, diminuição da temperatura de transição vítrea (T_g) ao incorporar PEG nas misturas, uma diminuição na força flexural de 71% e módulo de elasticidade de 80,4% para a mistura PLA/EVA/PEG/2,5QUI, bem como um aumento no alongamento na ruptura de 153% e 392% para a tenacidade ao impacto. Um comportamento semelhante foi observado para o PLA/EVA/20PEG e PLA/EVA/PEG/5,0QUI. O filme contendo QUI entre as fatias de pão também influenciou na redução da atividade de água, e reduziu cerca de 35% na contagem de bolores e leveduras nas fatias de pão. A

quitosana nas misturas com PLA/EVA/PEG apresentou potencial como agente antifúngico natural em embalagens de panificação.

Palavras-chave: Poli(ácido lático); Polietilenoglicol; Poli(etileno-co-acetato de vinila); Quitosana,; Antimicrobiano; Pão; Aplicações em embalagens.

Resumen
Este trabajo se centró en el desarrollo de envases activos biodegradables con mezclas de poli (ácido láctico) (PLA), poli (etileno-co-acetato de vinilo) (EVA), polietilenglicol (PEG) y quitosano (QUI). Se investigaron las características morfológicas térmicas y mecánicas de las mezclas, así como, al mismo tiempo, la actividad antifúngica del envase. Para evaluar la actividad antimicrobiana de las mezclas PLA/EVA/PEG/QUI, las muestras se insertaron entre rebanadas de pan sin conservantes para evaluar su vida útil. Al comparar entre PLA/EVA/PEG, mezclas de PLA/EVA/PEG/QUI y PLA puro fue posible evidenciar la miscibilidad parcial, la disminución de la temperatura de transición vítrea (T_g) al incorporar PEG en las mezclas, una disminución de la fuerza flexural del 71% y módulo de elasticidad del 80,4% a la mezcla PLA/ EVA/PEG/2.5QUI, así como un aumento del alargamiento a la rotura del 153% y del 392% a la tenacidad al impacto. Se observó un comportamiento similar a PLA/EVA/20PEG y PLA/EVA/PEG/5.0QUI. La película que contiene QUI entre las rebanadas de pan también influyó en la reducción de la actividad del agua y redujo aproximadamente un 35% en el recuento de mohos y levaduras en las rebanadas de pan. El quitosano en mezclas con PLA/EVA/PEG mostró potencial como agente antifúngico natural en envases de panadería.
Palabras clave: Poli(ácido láctico); Polietilenglicol; Poli(etileno-co-acetato de vinilo), Quitosano; Antimicrobiana; Pan; Aplicaciones de envasado.

1. Introduction

The development of biodegradable packaging with improved mechanical and barrier properties which will be useful for maintaining the safety and quality of foods is one of the main strategies currently researched (Dhall & Alam, 2020). One of the promising polymers for developing such kinds of packages is poly(lactic acid)(PLA). PLA is a biodegradable polymer derived from renewable sources, mainly starch and sugar, and has been used in many industrial applications due to its high hardness, modulus of elasticity and transparency (Arrieta, López, Ferrándiz, & Peltzer, 2013; Bhiogade, Kannan, & Devanathan, 2020; Guinault, Sollogoub, Domenek, Grandmontagne, & Ducruet, 2010; Jamshidian, Tehrany, & Desobry, 2013). On the other hand, for its application in food packages, it is necessary to modify some of its properties, mainly as regards its fragility attributed to its glass transition and hardness, and its high hydrolysis rate and low O_2 and CO_2 barrier properties (Armentano et al., 2013; Correa-Pacheco et al., 2020).

These PLA limitations might be eliminated to an extent, by blending/compounding with other polymers, plasticizers, or reinforcement fillers. As a proposed strategy for improving PLA properties, aiming at the production of food packages, is the development of PLA blends with poly(ethylene-co-vinyl acetate) (EVA) (Ma et al., 2012). The PLA and EVA blend seems an excellent option to deal with the main mechanical drawbacks, particularly low impact resistance and elongation at break (Aghjeh et al., 2015) while polyethylene glycol (PEG) improves the flexibility to the PLA blend (Choi, Choi, Han, Park, & Ha, 2013). PEG is a low-cost polymer when compared with poly(vinyl alcohol) (PVA) and hydroxylpropyl methylcellulose (HPMC), which acts as compatibilizer as well as a plasticizer and helps to reduce the brittleness of PLA. Generally, the hydroxyl group from PEG reacts with the carboxyl group of PLA. PEG molecules enter into the PLA macromolecules creating a physical interaction in the form of hydrogen bonding (Yuniarto et al., 2016).

Advanced packaging technologies have been playing a meaningful role in food industries. Food packages are required to be multi-functional in terms of chemical, physical, and biological alterations of the food in order to lengthen their shelf life, including the use of natural preservatives (Correa-Pacheco et al., 2020; Sengupta, Manna, Roy, & Das, 2020; Suwanamornlert et al., 2020).

Developments in the field of packaging have undergone a series of biosynthetic transformations considering the advance in the use of bio-based films and additives from renewable sources as opposed to the non-biodegradable films. Among the

additives used in packaging films, chitosan stands out for its biological and physical properties of biocompatibility, biodegradability, antimicrobial ability, and easy film forming ability (Wang, Qian, & Ding, 2018).

Chitosan (QUI) is a polysaccharide obtained by the deacetylation of chitin and has several applications due to its cost, excellent oxygen barrier properties, biodegradability, biocompatibility, antimicrobial activity and nontoxicity (Wang et al., 2018). The incorporation of QUI into PLA films increased its crystallization temperature and could influence the mechanical resistance and flexibility (Bonilla, Fortunati, Vargas, Chiralt, & Kenny, 2013). The importance of QUI consists in its film-forming ability (Elsabee & Abdou, 2013) combined with its antibacterial and antifungal properties (Rhim, Hong, Park, & Ng, 2006; Sébastien, Stéphane, Copinet, & Coma, 2006; Zhang, Li, & Liu, 2011). The chitosan antifungal activity takes place by the interaction between its cationic chain and the negatively charged macromolecules residues exposed on the fungi cellular surface, leading to intracellular electrolytes and other constituents' leakage (Muzzarelli et al., 2001). Danila Merino, Yamila Mansilla, Claudia Casalongué, & Vera Alvarez, 2016 developed PLA-Chitosan films with different concentrations of chitosan, and compatibilized with hexamethylenediisocyanate (HDI). The results showed that the compatibilizing agent reacts completely and the chitosan affects the thermal properties of the films decreasing their decomposition temperatures, glass transition temperature (T_g) and melting (T_m) and increase its crystallinity. The antimicrobial properties of the blends, tested against *Pseudomonas syringae pv tomato* DC-3000, showed that an increase in chitosan content, increases the antimicrobial effect.

Bakery products such as bread deteriorate rapidly due to loss of moisture and firmness in a process called 'aging' (Garcia, Bernardi, & Copetti, 2019; Miś, Krekora, Niewiadomski, Dziki, & Nawrocka, 2020). In addition, the products also undergo microbiological action due to the moisture in the bread, when slicing, wrapping the bread while it is still hot and/or storing the bread in a humid environment with a high temperature. Fungi can develop both inside and outside the bread, and the main factors that favor its development are the contamination of the dough during handling, an environment heavily loaded with spores, dirty or poorly sanitized equipment after use and exposure to environment for excessive time after cooking (dos Santos et al., 2016; Garcia et al., 2019).

Traditionally, antimicrobial compounds are incorporated to the food formulations or on their surfaces. Weak acids and their salts such as acetates, sorbates, benzoates, and propionates are widely used as preservative agents (Brul & Coote, 1999). Direct addition might result in excessive amounts of antimicrobial agents and consequently leads to food taste alteration (Uz & Altınkaya, 2011), besides being a risk to the consumer whereas elevated consumption of calcium propionate might lead to several health problems (Phechkrajang & Yooyong, 2017).

Sorbic acid is used as conservative in a range of food products (bakery, dairy, meat, fruits, vegetables, and beverages). The allowed levels depend on the kind of product and its country of origin, but the maximum content is usually 0.2%. Higher concentrations are allowed for packages or surfaces treatments. Several scientific studies were carried out and demonstrated sorbic acid and its salts do not represent any kind of health hazard (Hsieh et al., 2012; Ling et al., 2015). Sorbic acid has been used in fermented bakery products in combination with calcium propionate (Thomas & Delves-Broughton, 2014). Calcium propionate has been widely utilized as a conservative for baking products, although it can also be added to non-alcoholic beverages, fresh dough, pizza dough, puddings, gelatins, jams, among other food products. Propionic acid and its salts, sodium and calcium propionate are approved in the United States as GRAS substances (Generally Acknowledged as Safe) for food use.

Calcium propionate is listed as number E282 in the Codex Alimentarium, and the recommended dosage is not fixed, however following the Good Manufacturing Practices (GMP), the concentrations may not exceed 0.4% (FAO/WHO, 1995). The USA Food and Drug Administration (FDA/U.S.) recommends a daily limit of calcium propionate intake to 1 mg.(kg.d)$^{-1}$, however, in view of its indiscriminate consumption, there may have an extrapolation in the daily recommended amount (Phechkrajang & Yooyong, 2017), and in accordance with researchers high consumption might lead to several health issues. Propionate conservatives might contribute or be a causative agent of hyperactivity (Swain, Soutter, Loblay, & Truswell, 1985),

visual hallucinations (Shuaib et al., 2012), irritability, unquietness, and lack of attention and sleep disorders in children (Al-Owain et al., 2013; Dengate & Ruben, 2002; Pena & Burton, 2012).

PLA as described above exhibits properties of interest to the field of packaging. However, its high cost and fragility make it difficult to apply it in the production of films, which has also been previously justified. Blended PLA with other polymers enables the change of tenacity, however, when mixed with 10, 20 and 30% by weight of chitosan, the films showed improvements in mechanical properties, as described by (Claro et al., 2016), when reported an increase of 4.7% in elongation at break and the potential application of this films in packaging fields.

Therefore, there is a worldwide trend for the incorporation of chemical products and antimicrobial agents into packages aiming at rendering them active and biodegradable. (Heras-Mozos et al., 2019) developed active antifungal packaging by incorporating garlic extract and bread aroma in a polymeric coating on a polyethylene (PE) bag for the preservation of natural sliced pan loaf without synthetic chemical preservatives. (Srisa & Harnkarnsujarit, 2020) showed that films from trans-cinnamaldehyde incorporated into poly(lactic acid) and poly(butylene adipate-co-terephthalate) effectively inhibited the microbial growth of bacteria and fungi in the bread for more than 21 days at 30 °C. (Suwanamornlert et al., 2020) concluded the PLA and poly(butylene-succinate-co-adipate)(PBSA) films containing thymol are promising biodegradable antifungal packaging materials for increasing the shelf life of bread and potentially other food products.

A considerable advantage of antimicrobial packaging over the direct addition of preservatives is the gradual releasing of the active agent on the surface of food during storage and distribution. Hence, the main objective of this work was to develop a biodegradable packaging blend with antifungal activity by mixing PLA with poly(ethylene-co-vinyl acetate) (PVA), polyethylene glycol (PEG), and chitosan (QUI) for bakery products application. For this purpose, the morphological, thermal and mechanical characteristics of the packaging and the antimicrobial activity of chitosan exposed to bread were evaluated.

2. Methodology

The PLA used in this study was a commercial-grade supplied by Nature-Works PLA 3251D, with a density of 1.24 g.cm^{-3} and melt flow index of 35 g.10 min^{-1} (190 °C, 2.16 kg), supplied by Cargill (Brazil). The EVA used was also a commercial-grade (HM150), containing 20% of vinyl acetate, supplied by Braskem (Brazil), which has a melt flow index of 150 g.10 min^{-1} (190 °C, 2.16 kg) and density of 0.940 g.cm^{-3}. PEG with Mn of 20000 g.mol^{-1} and chitosan (75−85% degree of deacetylation) were purchased from Sigma Aldrich (USA). Ingredients for bread making were purchased at a local market (Caxias do Sul, RS, Brazil).

Blends of PLA (80% wt), EVA (20% wt), and PEG (20 phr – parts per hundred resin - blend), with the addition of chitosan (2.5 and 5.0 phr) were prepared and named PLA/EVA/PEG/2.5QUI and PLA/EVA/PEG/5.0QUI, respectively. A blend without the addition of chitosan (PLA/EVA/20PEG) was prepared and used as control. The concentration of PLA, EVA, PEG, and QUI were chosen according to previous studies (Bonilla et al., 2013; Choi et al., 2013; Hassouna et al., 2011; Ma et al., 2012). PLA and EVA pellets were dried in an oven at 60 °C for 12 h before processing in order to eliminate possible absorbed water on the surface of the particles.

The blends formulations were manufactured by using a co-rotating twin-screw extruder (MH model, L/D 32, MH Equipment, Brazil), at a speed of 150 rpm. The temperature profile was Z1: 140, Z2: 155, Z3: 165, Z4: 170, Z5: 175, Z6: 170, Z7: 165, and Z8: 175 °C with an open matrix. The extruded material was crushed in a knife mill (model MA 580, MARCONI, Brazil). Test specimens were prepared by injection-molding (LH15080, HIMACO, Brazil) using previously dried powder of the blends according to ASTM D790-03, and used for further characterization. The blends were manufactured by compression

molding in a PH5 machine model (SCHULZ, Brazil) to produce films of 100 mm × 100 mm × 2 mm. Molding conditions were set at 170 ˚C for 5 min and 11.8 MPa.

Scanning Electron Microscopy (SEM) was used to characterize the samples cryogenic fractures morphology. Images of the cross-section were obtained by cryogenically fracturing the injection-molded test samples. The surface of the fractures were sputter-coated with gold and observed using a Shimadzu SSX−550 Superscan (Japan) apparatus operating at 15 kV.

Thermogravimetric analysis (TGA) was used to measure the thermal weight loss of the blends in a Shimadzu TGA-50 instrument (Japan). The samples were heated from room temperature to 800 ˚C at the heating rate of 10 ˚C.min^{-1} under nitrogen flow (50 mL.min^{-1}).

Differential scanning calorimetry (DSC) was used to determine the melting (T_m), crystallization (T_{cc}), and glass transition temperatures (T_g), using a Shimadzu DSC-60 (Japan) instrument with a heating rate of 10 ˚C. min^{-1} at the temperature range from 25 to 220 ˚C, under 50 mL.min^{-1} nitrogen flow rate in a single run. The flexural properties were measured according to ASTM D790, using a universal test machine (DL-300 model, EMIC, Brazil) with a load cell of 100 kgf and speed of 1.5 mm.min^{-1}. All the tests were performed at ambient temperature.

The bread preparation without preservatives was carried out as described by (Pinilla, Thys, & Brandelli, 2019), with some modifications, which are briefly described here. Bread was manufactured at Cereal Laboratory at the University of Caxias do Sul (Caxias do Sul, Brazil) and all the bread ingredients were obtained from local bakery products suppliers. The loaf bread recipe included 1000 g wheat flour free of antifungal compounds (Orquidea, Caxias do Sul, Brazil), 45 g sucrose, 40 g vegetal oil, 20 g baker yeast, 20 g NaCl, 3 g reinforcing emulsifier based on esters of fatty acids mono- and diglycerides with tartaric diacetyl acid and 600 mL tap water. The ingredients were kneaded for 5 min, and then the bread dough was maintained resting for 10 min and cut into pieces of 165 g before the fermentation process at 30 ˚C, for 90 min in a climatic chamber (CC1000 model, G. PANIZ, Brazil). Baking process was performed at 160 ˚C for 30 min in a deck oven (FTT-80-G, Tedesco, Brazil). The loaves were kept for 60 min on cooling racks at room temperature. Each loaf was cut into three slices with approximately 10 cm high by 3 cm length.

Commercial breads containing chemical preservative (Pullmann, Brazil) were purchased from a local store and cut into three slices with approximately 10 cm high by 3 cm length. According to the manufacturer's information, it contained wheat flour enriched with iron and folic acid, soy vegetable oil, gluten, salt, vinegar, emulsifiers, mono- and diglycerides of fatty acids, calcium stearoyl-2-lactyl lactate, and polysorbate. The manufacturer did not inform the amount of ingredients and additives used in the formulation.

The previously cut slices of bread with and without preservative were exposed to the blends (PLA/EVA/20PEG, PLA/EVA/PEG/2.5QUI, and PLA/EVA/PEG/5.0QUI films), according to the procedure described by (Kechichian, Ditchfield, Veiga-Santos, & Tadini, 2010), with some modifications, which are briefly described here. The developed films were placed between two bread slices and packaged into 0.07 mm thickness polypropylene (PP) bags. The sets were stored at room temperature (23 ± 2 ˚C) during 7 days without humidity control, aiming to simulate possible storage conditions and their effects on the product.

The counting of filamentous fungi and yeasts was performed according to the Official Methods of Analysis of Association of Official Analytical Chemistry (AOAC), by the method 997.02. All results were expressed as Colony Forming Units per gram (CFU. g^{-1}), before and after 7 days of the storage. The experiments were performed in triplicate.

The moisture content was evaluated according to the Official Methods of the AOAC, by method 925.09 (Association of Official Analytical Chemistry, 1997). The samples were placed in a drying oven with air circulation at 105 ˚C for 24 h (520 model, Fanem, Brazil). Water activity (aw) of the slices of bread was measured on the first day, one hour after the cooking, and after seven days of exposure to the films. For the analysis, a portion of the central region of the sliced bread (crumbs) was

measured using an activity analyzer (Decagons Aqua Lab, Series 3TE, Pullman, USA) at 25 ± 2 ˚C. The experiments were performed in triplicate.

The texture profile analysis (firmness and elasticity) of the sliced bread was carried out using a TA.XT2 texturometer (Stable Micro Systems, UK), in agreement with the methodology developed by the American Institute of Baking (American Institute of Baking 2014). The experiments were performed in triplicate, before and after seven days.

Bread crumb color determination was performed in duplicate by the CIE method (L*b*a* system), using a CR 400 Chroma Meter colorimeter (Konica Minolta, Japan). The color was measured using three parameters: L*, which varies from 100 (white) to zero (black), b*, which varies from blue (negative) to yellow (positive) and a*, which varies from green (negative) to red (positive). The experiments were performed in triplicate, before and after seven days.

Results are presented as average ± SD. Statistical analysis was carried out by ANOVA test followed by Tukey test, using SSPS software package 16.0 (IBM SPSS Statistics, Inc., Chicago, IL, USA), and differences between groups were considered significant at $p < 0.05$.

3. Results and Discussion

The morphological properties of the fracture surfaces of PLA, PLA/EVA/20PEG, PLA/EVA/PEG/2.5QUI, and PLA/EVA/PEG/5.0QUI polymer blends were investigated by SEM, and shown in Figure 1. Pure PLA exhibits a surface with a few protrusions, similar to the morphology observed by (Sungsanit, Kao, Bhattacharya, & Pivsaart, 2010) and (Abdelwahab et al., 2012). PLA/EVA/20PEG blend (Figure 1b) showed EVA domains characterizing the immiscibility of the blend, as evidenced by (Ma et al., 2012). PEG did not show phase separation. Based on the polar character of PLA and PEG, the blend presented partial miscibility. Studies reported by (Sungsanit et al., 2010) confirm the statement of miscibility of PLA/PEG blends with 5.0 to 10 % wt of PEG and molar weight of 1000 g.mol^{-1}.

QUI addition to the polymer blends promoted irregularities and discontinuities on the matrix (Bonilla et al., 2013). Micrographs of PLA/EVA/PEG/2.5QUI and PLA/EVA/PEG/5.0QUI blends showed a layered structure with size variation from 100 to 500 µm (Figure 1 c, d). This might be attributed to fiber agglomeration. (Correlo et al., 2005) observed similar results for QUI/PLA blends (50/50% wt), also indicating the formation of agglomerated and irregularly distributed chitosan fibers. The formation of these layers might lead to significant decreases in reinforcement efficiency.

The effect of PEG and QUI incorporation on the thermal stability of PLA/EVA blends was studied using the thermogravimetric analysis, as shown in Figure 2 (a) and (b). The weight loss of PLA occurred in a single step starting at 339 ˚C, at a T_{max} value of 356 ˚C, and ending at 374 ˚C, with a weight loss of 96.5%. (Zhao et al., 2010) reported similar behavior. In the case of PLA/EVA blends, two weight loss steps are observed. The first peak occurred at around 362 ˚C and was attributed to the decomposition of the PLA while the second peak observed at around 466 ˚C, corresponded to the degradation EVA at high temperature and is associated with the loss of unsaturated poly(ethylene-co-acetylene) (Moura, Nogueira, Bounor-Legare, & Machado, 2012).

The TGA curve for PLA/EVA/20PEG blend involves three zones of weight loss. The first weight loss between 364−395 ˚C with T_{max} at 378 ˚C, of 74. 6%, was due to the decomposition of the PLA/EVA matrix, related to the production of PLA carboxyl groups and scission of the EVA acetate groups. The second between 424−434 ˚C with a weight loss of 4.7%, and third event occurring between 472−495 ˚C, with 7.2% of weight loss, these events are correlated to PEG degradation. The incorporation of PEG shifts systematically the T_{onset} and T_{max} of PLA/EVA/20PEG blends to a higher temperature when compared to pure PLA. This shift was due to the higher molar weight (Mn=20000 g.mol^{-1}) of PEG, which contributes to an increase in thermal stability of the blends.

Figure 1 - SEM Micrographs of the cryogenic fracture based on test specimens for flexural strength of original magnitude 2000x (a)PLA, (b) PLA/EVA/20PEG, (c) PLA/EVA/PEG/2.5QUI, (d) PLA/EVA/PEG/5.0QUI.
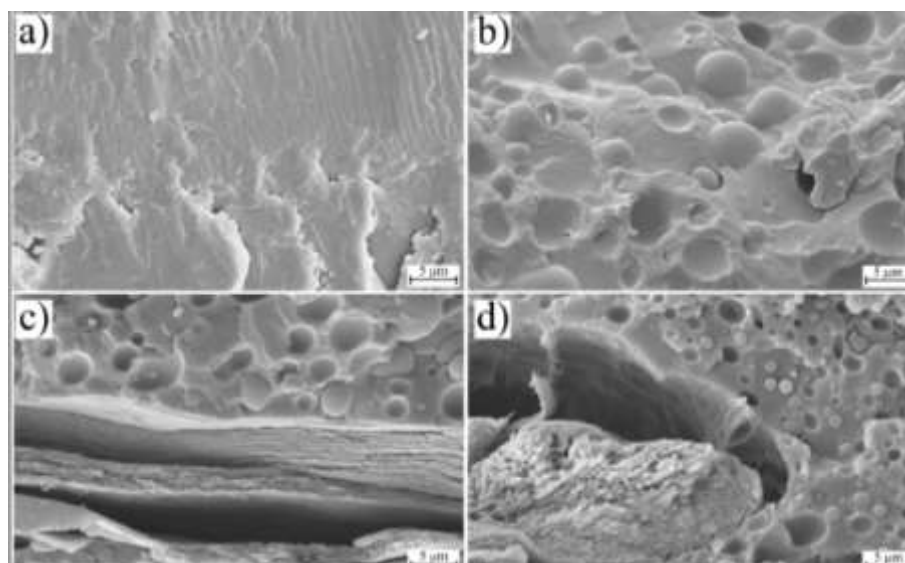
Source: Authors.

The incorporation of QUI had no significant effect on the T_{onset} and T_{max} values of the PLA/EVA/PEG/2.5QUI and PLA/EVA/PEG/5.0QUI blends compared to the PLA/EVA/20PEG, as shown in Figure 2b. For QUI containing blends, three weight loss steps were observed. The first one with T_{max} at 371.8 ˚C (67.7%) for PLA/EVA/PEG/2.5QUI blend and 367.3 ˚C (68.0%) for PLA/EVA/PEG/5.0QUI blend and these weight losses were attributed to the EVA acetate groups deacetylation or breaking, yielding a gaseous acetic acid and forming double bonds (C=C) (Figure 2a) (Ma et al., 2012). Even though the thermal behavior of PLA/EVA/PEG/2.5QUI and PLA/EVA/ PEG/5.0QUI blends is similar to PLA/EVA/20PEG, a shift to higher decomposition temperatures was observed, which its increasing is proportional to QUI. A similar behavior was seen in studies performed by (Bonilla et al., 2013) utilizing contents of 5 and 10% QUI blended with PLA. The incorporation of EVA, PEG, and QUI into the PLA shifts the degradation temperatures towards higher values, promoting an increase in PLA thermal stability.

Figure 2 – Curve and temperatures for TGA of (a) PLA, PLA/EVA and (b) PLA/EVA/20PEG, PLA/EVA/PEG/2.5QUI, PLA/EVA/PEG/5.0QUI blends.
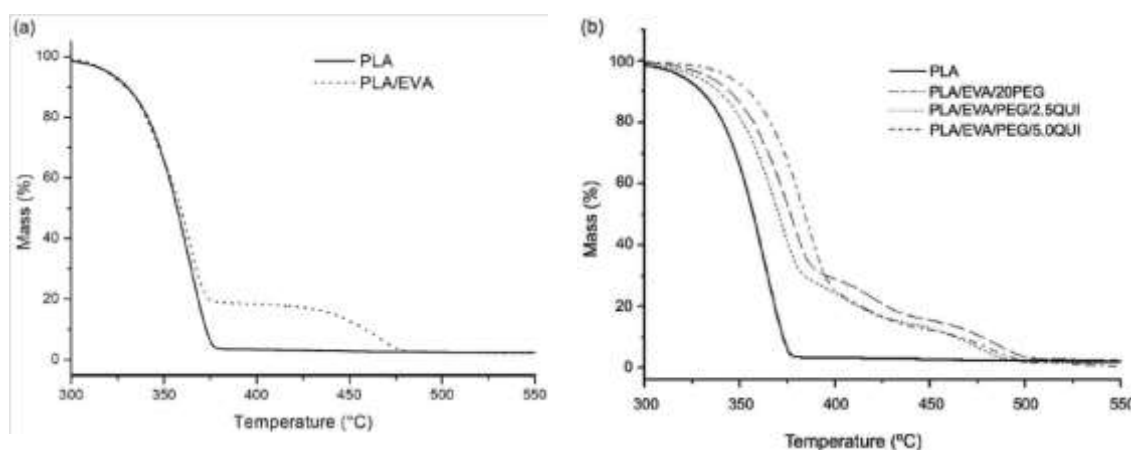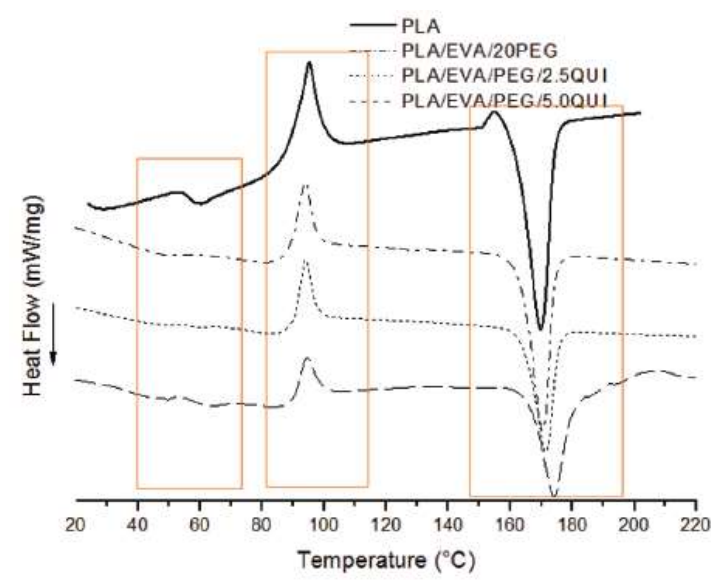

Source: Authors.

The first DSC heating curves of the pure PLA and the blends are shown in Figure 3. The PLA showed a glass transition (T_g) at 60.4 ˚C, an exothermic crystallization peak (Tcc) at 95.2 ˚C, and an endothermic melting peak (T_m) at 169.8 ˚C. The T_g value of the PLA/EVA blend (data not shown in the figure) did not show any variation in value than that of pure PLA. However, the addition of PEG led to a shift in T_g of the PLA/EVA/20PEG blend to a lower temperature ($T_g = 49$ ˚C) when compared with pure PLA. This shift was attributed to the PEG plasticizing effect. The decrease in T_g of the blend implies more mobility of the polymer segments and might be explained by the increase of PLA chains disentanglement caused by the presence of PLA-PEG chains which lead to higher free volume (Hassouna et al., 2011; Ma et al., 2012).

The DSC curves of PLA/EVA/PEG/2.5QUI and PLA/EVA/PEG/5.0QUI blends have also shown a shift in T_g to lower values, near 51 and 55 ˚C, respectively. The difference in T_g values between the QUI-containing blends is negligibly small. The incorporation of QUI into polymer blends did not significantly influence the glass transition temperature of the blend. The reduced T_g values of the polymer blends when compared to PLA, improved chain flexibility making them attractive and useful for food packaging applications. This difference might also be attributed to moisture loss that is frequently observed for several polysaccharides, such as cellulose and chitin derivatives (Ganji & Abdekhodaie, 2008). The loss of moisture can increase the digestibility of the mixture, justifying the small difference observed.

Melting temperature (T_m) of the PLA/EVA/20PEG and PLA/EVA/PEG/2.5QUI blends mostly did not change when compared to the pure PLA. However, the addition of 5.0 phr of QUI in PLA/EVA/PEG/5.0QUI blend led to a slightly shift in T_m to a higher temperature. This increase can be associated with a slight reduction in PLA molar mass during extrusion and the ability of QUI to act as a nucleating agent, promoting quicker PLA crystallization in combination with the crystallinity of the materials (Torres-Huerta, Palma-Ramírez, Domínguez-Crespo, Del Angel-López, & De La Fuente, 2014).

Figure 3 - DSC Curves and characteristic transitions of PLA, PLA/EVA/20PEG, PLA/EVA/PEG/2.5QUI, and PLA/EVA/PEG/5.0QUI blends.

Source: Authors.

The mechanical properties of PLA, PLA/EVA/20PEG, PLA/EVA/PEG/2.5QUI, and PLA/EVA/PEG/5.0QUI blends are shown in Table 1. The mechanical behavior of the pure PLA showed characteristics of glassy polymers with low deformation at break (1.87%). The flexural strength and elastic modulus of the blends were significantly reduced by the addition of PEG and

QUI, but the elongation at break increased in value. This phenomenon was also reported by (Claro et al., 2016). Blends of PLA with 10 wt% of chitosan showed improvements in mechanical properties as an increase of 4.7 % in elongation at break, and proved to be the best candidate for application in packaging. (Danila Merino et al., 2016) have been identified the same behavior reported in this study, using PLA-Chitosan films compatibilized with hexamethylenediisocyanate (HDI).

The PLA/EVA/20PEG blend had a higher value of elongation at break than pure PLA. As already observed in DSC results, the incorporation of PEG in the PLA/EVA/20PEG blend has shown a significant effect on the glass transition temperature of PLA, decreasing its value due to the plasticizing effect of PEG. As expected, adding PEG to the blends leads to an increase in the elongation at break due to the reduction in molecular stiffness and consequent increasing in the deformation capacity of the polymer chains. Similar results have been observed by (Byun, Kim, & Whiteside, 2010). The addition of PEG into the PLA film increased elongation and reduced brittleness by decreasing T_g of the blend. It was also observed that the addition of QUI to the blends had no significant effect on the elongation at break when compared with PEG. However, the presence of QUI promoted an increase at impact toughness probably due to the polar interactions between ester functional groups of PLA and amine groups of chitosan. The chitosan appears to act as stress concentrators in the polymer blends enhancing the impact toughness. The stress concentrator effect attributed to chitosan is supported by the study by (Tanase & Spiridon, 2014) in the PLA/Keratin mixture with the addition of 2 and 4% by mass of keratin. The authors observed an increase in the impact resistance property explained by the keratin structure acting as a stress concentrator in the polymer matrix, reducing the energy at the beginning of the break. The best result of properties of interest for packaging was obtained for blend PLA/EVA/PEG/2.5QUI. The concentration of the 2.5 QUI, appear to be the optimum concentration for the blend, above this, the higher content of QUI (PLA/EVA/PEG/5.0QUI) appears to have acted negatively to the impact toughness, reducing its value, probably due to the agglomeration and the irregularly distribution of the chitosan fibers, as observed by SEM.

For application in films, in addition to the melt index, it is important that the polymer has flexibility, and this characteristic is opposite to the stiffness identified in the modulus of elasticity. Another aspect to be considered is the toughness, energy absorbed before the fracture and this property is a sum not only of tensile strength, bending but also of the deformation that the material presents. PLA, for being a polymer considered rigid, given its T_g and high modulus of elasticity. For use in films, it needs to become more tenacious, and this was observed in this study by the incorporation of EVA and PEG, increasing deformation and impact resistance, characteristics that prove the toughness proposed in the objective of the study.

Table 1. Flexural strength, elasticity modulus, elongation at break, and impact toughness of pure PLA, PLA/EVA/20PEG, PLA/EVA/PEG/2.5QUI, and PLA/EVA/PEG/5.0QUI blends.

Samples	Flexural strength (MPa)	Elasticity modulus (MPa)	Elongation at break (%)	Impact toughness (J m⁻¹)
PLA	60.8 ± 3.6[a]	3354.7 ± 288.8[a]	1.87 ± 0.15[a]	26.6 ± 2.1
PLA/EVA/20PEG	24.4 ± 1.7[b]	931.1 ± 90.3[b]	4.66 ± 0.13[b]	27.7 ± 3.6
PLA/EVA/PEG/2.5QUI	17.6 ± 1.4[b]	658.8 ± 69.2[b]	4.74 ± 0.25[b]	130.9 ± 63.2
PLA/EVA/PEG/5.0QUI	18.8 ± 1.3[b]	762.5 ± 65.1[b]	4.71 ± 0.15[b]	97.8 ± 64.6

Different letters (a-b) indicate significant differences ($p < 0.05$).
Uncertainties refer to the standard deviation of the mean, as obtained from three independent measurements.
Source: Authors.

As shown in Table 2, the moisture presented significant difference ($P < 0.05$) between different bread (commercial and manufacture) and during storage (bread manufacture). In commercial bread, the moisture content remained practically unaltered after seven days of storage, probably due to the more quantity of emulsifiers, which increases water absorption ability. However, in the manufactured bread was observed a reduction in the moisture after seven days of storage, suggesting the syneresis

phenomena occurred similarly for all the treatments. The moisture content is associated with the retrogradation index, which indicates the trend of bread dryness. Since it is closely related to syneresis caused by starch retrogradation, this process might be linked to the re-association of amylopectin chains that causes the system water outlet (Miś et al., 2020).

A reduced water activity in the breadcrumbs was already expected to occur throughout the storage period by the migration of water from the breadcrumbs towards the outer medium (Licciardello, Cipri, & Muratore, 2014). Besides this phenomenon, the QUI-containing film among the bread slices has also influenced the water activity reduction, with lower values as a function of QUI concentration in the films. Water migration from the crumb towards the QUI-containing films can be explained by the high QUI hydrophilicity because of hydroxyl and amino groups in the polymer chain (van den Broek, Knoop, Kappen, & Boeriu, 2015).

The texture (hardness) is an important quality indicator of bread. Results obtained for texture (firmness and elasticity) evidenced structural changes in the breads after seven days storage, mainly in the manufactured breads. However, it was observed that adding chitosan into the films (2.5 and 5.0 phr) did not significantly affect the hardness comparing the different treatments. When the statistic is applied, the same treatment in the time span of 1 to 7 days, a significant difference occurs. The texture characteristics are related with gelatinized starch recrystallization (retrogradation) during the storage. In order to slow this phenomenon, enzymes mixes (α-amylases and lipases) and emulsifiers are added to the bread-manufacturing step. Enzymes and emulsifiers reinforce the dough physical properties, increasing gases trapping, besides improving the conservation of the bread softness for a longer period (León, Durán, & de Barber, 2002). The firmness and elasticity values in the commercial bread also showed a significant change ($P < 0.05$); however, the variation was smaller compared to the manufactured breads, possibly due to the higher amount of enzymes and emulsifiers added.

Table 2. Analysis of moisture, water activity and texture (Firmness and elasticity) of the commercial and fresh sliced breads before and after exposure to PLA/EVA/20PEG, PLA/EVA/PEG/2.5QUI and PLA/EVA/PEG/5.0QUI polymer blends.

Parameters	Days	Commercial bread[†]	PLA/EVA/20PEG	PLA/EVA/PEG/2.5QUI	PLA/EVA/PEG/5.0QUI
Moisture (%)	1	33.70 ± 0.60[a]	31.70 ± 0.30[b]	31.70 ± 0.30[b]	31.70 ± 0.30[b]
	7	32.60 ± 0.20[ab]	27.30 ± 0.40[c]	27.35 ± 0.20[c]	26.90 ± 0.10[c]
Water Activity	1	0.958 ± 0.003[a]	0.931 ± 0.012[b]	0,931 ± 0.012[b]	0.931 ± 0.012[b]
	7	0.949 ± 0.003[ab]	0.919 ± 0.006[bc]	0,909 ± 0.008[c]	0.904 ± 0.021[c]
Firmness (N)	1	3.61 ± 0.13[c]	5.26 ± 0.52[b]	5,26 ± 0.52[b]	5.26 ± 0.52[b]
	7	4.81 ± 0.27[b]	9.42 ± 0.55[a]	9.81 ±0.89[a]	11.11 ± 1.10[a]
Elasticity (%)	1	58.00 ± 0.71[a]	54.22 ± 0.98[b]	54.22 ± 0.98[b]	54.22 ± 0.98[b]
	7	56.00 ± 0.66[a]	49.04 ± 0.61[c]	48.73 ± 0.53[c]	47.94 ± 0.58[c]

Different letters (a-c) indicate significant differences ($p < 0.05$). Each set of data was determined as an average of three replicates.
[†] Commercial bread exposed to PLA/EVA/20PEG film
Source: Authors.

The values of the chromatic coordinate L* did not significantly affect during the storage process (Table 3). High values of L* indicate greater light reflectance, translating into light colored breads. The dimming is a characteristic phenomenon caused by the loss of moisture (Licciardello et al., 2014). For a* chromaticity, a significantly increase in values was observed after 7 days of storage. To the b* chromaticity, there was a slight, non-significant drop in the values of samples that remained in contact with the polymer mixtures containing chitosan. Different behavior was observed for the control sample, where it was evidenced significant increase in this parameter, translating into a more yellowish. Variations in the values of a* and b* may be related to the degree of aeration (porosity of the mass), the moisture content of the samples, in addition to the effect of chitosan.

However, the results of the color changes observed in the breads might be more attributed to the presence of natural pigments in the wheat flour, which can oxidize during the storage process (Sopiwnyk et al., 2020) than blends.

Table 3. Colorimetric analysis of the bread crumbs before and after seven days of the exposure to PLA/EVA/20PEG, PLA/EVA/PEG/2.5QUI, and PLA/EVA/PEG/5.0QUI films.

Parameters	Days	PLA/EVA/20PEG	PLA/EVA/PEG/2.5QUI	PLA/EVA/PEG/5.0QUI
L*	1	82.90 ± 0.50^a	82.86 ± 0.48^a	82.86 ± 0.48^a
	7	80.61 ± 0.48^a	80.87 ± 0.50^a	79.39 ± 0.56^a
a*	1	0.21 ± 0.08^c	0.21 ± 0.08^c	0.21 ± 0.08^c
	7	0.54 ± 0.02^a	0.37 ± 0.05^b	0.33 ± 0.08^b
b*	1	16.56 ± 0.05^b	16.56 ± 0.05^b	16.56 ± 0.05^b
	7	17.38 ± 0.22^a	16.06 ± 0.57^b	16.20 ± 0.54^b

Different letters (a-c) indicate significant differences ($p < 0.05$)
Source: Authors

The number of viable colonies of filamentous fungi and yeast on the bread slices on the first day of storage was below 1500 $CFU.g^{-1}$ (Figure 4). On the other hand, after seven days of storage, the colonies on the bread slices' surfaces increased considerably (between 4.200 and 8.900 $CFU.g^{-1}$). According (Garcia et al., 2019) fungal deterioration in bread is strongly related to the high-water activity and slightly acidic pH of this product that restricts the growth of other microorganisms. In this study, the pH values of the breads were not determined. In addition, this product is an excellent source of carbohydrates and has a porous structure that facilitates the fixation of fungal mycelia and provides adequate support of oxygen for spore's multiplication (Garcia et al., 2019).

Figure 4 - Number of viable colonies of mold and yeast in the bread slices exposure to PLA/EVA/20PEG, PLA/EVA/PEG/2.5QUI and PLA/EVA/PEG/5.0QUI films.
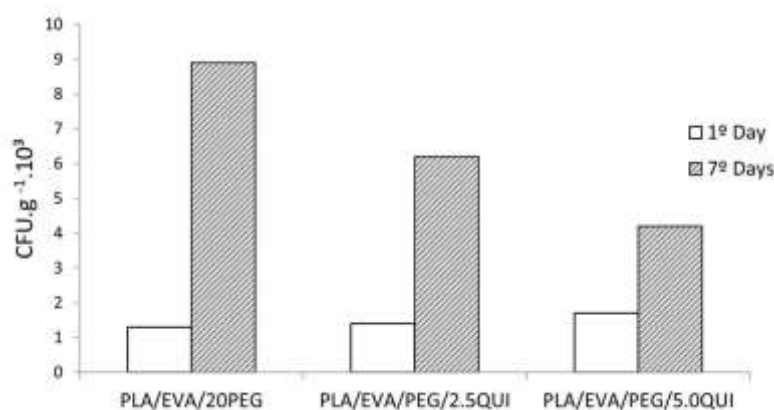
Source: Authors.

However, for the bread slices in contact with PLA/EVA/PEG/2.5QUIand PLA/EVA/ PEG/5.0QUI films, the mold and yeasts CFU counting was lower as compared to the control film (PLA/EVA/20PEG). Besides, the best results were observed for breads kept in contact with the polymer blend containing 5.0 phr QUI, confirming the QUI antifungal effect. Similar results was observed by (Claro et al., 2016), in blends of PLA containing 10, 20 and 30% by weight of chitosan and (Wang et al., 2018) with the potential use of chitosan with antimicrobial action.

In the commercial bread was determined lower number of viable colonies of molds and yeasts ($\cong 1.0 . 10^2$), data not shown. This result might be due to the presence of sorbic acid and calcium propionate inside the formulation, these known preservatives.

The results obtained in this work indicated the polymer PLA/EVA/PEG/5.0QUI blend showed approximate reduction of 35% in the molds and yeasts counting. Besides, the use of biodegradable packing with incorporation of natural active ingredients attends an emerging demand of the food industry, since researches suggest healthiness and environmentally friendly packaging as consumer aspiration.

4. Conclusions

The EVA blending to PLA by extrusion led to the formation of immiscible blends of PLA/EVA and partially miscible blends by incorporating PEG. By comparing the properties of PLA to the blends it was possible to observe a decrease in flexural strength of 71.0% and elasticity modulus of 80.4% to the mixture with incorporation the 20 phr PEG and 2.5 phr QUI(PLA/EVA/PEG/2.5QUI) besides an increase in elongation at break of 153% and 392% to impact toughness. These properties achieved with the blends have been promoting the PLA properties for packages production. The chitosan also showed potential as a natural antifungal agent in bakery packing once the compression molded films results placed between bread slices showed a reduction of approximately 35% in molds and yeast CFU counting. Bread exposure to the PLA/EVA/PEG/2.5QUI and PLA/EVA/PEG/5.0QUI polymer blends caused an inhibitory effect on fungal growth, with the 5.0 phr QUI sample presenting the lower development of molds and yeasts. As regards the remaining physical-chemical features, no statistical differences were observed in bread features when compared to the control sample after exposure to the polymer blends. These differences are more evident when it is considered that commercial bread has preservatives in its constitution, in terms of the bread produced for the study. Thus, the incorporation of chitosan in biodegradable polymers provides the development of active packaging, which can improve the quality and safety of packaged foods, reducing the use of preservatives, which was presented in this study.

Acknowledgments

References

Abdelwahab, M. A., Flynn, A., Chiou, B.-S., Imam, S., Orts, W., & Chiellini, E. (2012). Thermal, mechanical and morphological characterization of plasticized PLA-PHB blends. *Polym Degrad Stabil*, *97*, 1822–1828. https://doi.org/https://doi.org/10.1016/j.polymdegradstab.2012.05.036

Aghjeh, M. R., Nazari, M., Khonakdar, H. A., Jafari, S. H., Wagenknecht, U., & Heinrich, G. (2015). In depth analysis of micro-mechanism of mechanical property alternations in PLA/EVA/clay nanocomposites: A combined theoretical and experimental approach. *Mater Design*, *88*, 1277–1289. https://doi.org/https://doi.org/10.1016/j.matdes.2015.09.081

Al-Owain, M., Kaya, N., Al-Shamrani, H., Al-Bakheet, A., Qari, A., Al-Muaigl, S., & Ghaziuddin, M. (2013). Autism Spectrum Disorder in a Child with Propionic Acidemia. In G. Brown, E. Morava, V. Peters, K. M. Gibson, & J. Zschocke (Eds.), *JIMD Reports - Case and Research Reports* (pp. 63–66). https://doi.org/https://doi.org/10.1007/8904_2012_143

Armentano, I., Bitinis, N., Fortunati, E., Mattioli, S., Rescignano, N., Verdejo, R., & Kenny, J. M. (2013). Multifunctional nanostructured PLA materials for packaging and tissue engineering. *Prog Polym Sci*, *38*, 1720–1747. https://doi.org/https://doi.org/10.1016/j.progpolymsci.2013.05.010

Arrieta, M. P., López, J., Ferrándiz, S., & Peltzer, M. A. (2013). Characterization of PLA-limonene blends for food packaging applications. *Polymer Testing*, *32*(4), 760–768. https://doi.org/10.1016/j.polymertesting.2013.03.016

Bhiogade, A., Kannan, M., & Devanathan, S. (2020). Degradation kinetics study of Poly lactic acid(PLA) based biodegradable green composites. *Materials Today: Proceedings*, *24*, 806–814. https://doi.org/10.1016/j.matpr.2020.04.389

Bonilla, J., Fortunati, E., Vargas, M., Chiralt, A., & Kenny, J. M. (2013). Effects of chitosan on the physicochemical and antimicrobial properties of PLA films. *J Food Eng*, *119*, 236–243. https://doi.org/https://doi.org/10.1016/j.jfoodeng.2013.05.026

Brul, S., & Coote, P. (1999). Preservative agents in foods: Mode of action and microbial resistance mechanisms. *Int J Food Microbiol*, *50*, 1–17. https://doi.org/https://doi.org/10.1016/S0168-1605(99)00072-0

Byun, Y., Kim, Y. T., & Whiteside, S. (2010). Characterization of an antioxidant polylactic acid (PLA) film prepared with α--tocopherol, BHT and polyethylene glycol using film cast extruder. *J Food Eng*, *100*, 239–244. https://doi.org/https://doi.org/10.1016/j.jfoodeng.2010.04.005

Choi, K., Choi, M.-C., Han, D.-H., Park, T.-S., & Ha, C.-S. (2013). Plasticization of poly(lactic acid) (PLA) through chemical grafting of poly(ethylene glycol) (PEG) via in situ reactive blending. *Eur Polym J*, *49*, 2356–2364. https://doi.org/https://doi.org/10.1016/j.eurpolymj.2013.05.027

Claro, P. I. C., Neto, A. R. S., Bibbo, A. C. C., Mattoso, L. H. C., Bastos, M. S. R., & Marconcini, J. M. (2016). Biodegradable Blends with Potential Use in Packaging: A Comparison of PLA/Chitosan and PLA/Cellulose Acetate Films. *Journal of Polymers and the Environment*, *24*(4), 363–371. https://doi.org/10.1007/s10924-016-0785-4

Correa-Pacheco, Z. N., Black-Solís, J. D., Ortega-Gudiño, P., Sabino-Gutiérrez, M. A., Benítez-Jiménez, J. J., Barajas-Cervantes, A., & Hurtado-Colmenares, L. B. (2020). Preparation and characterization of bio-based PLA/PBAT and cinnamon essential oil polymer fibers and life-cycle assessment from hydrolytic degradation. *Polymers*, *12*(1), 1–32. https://doi.org/10.3390/polym12010038

Correlo, V. M., Boesel, L. F., Bhattacharya, M., Mano, J. F., Neves, N. M., & Reis, R. L. (2005). Properties of melt processed chitosan and aliphatic polyester blends. *Materials Science and Engineering A*, *403*, 57–68. https://doi.org/10.1016/j.msea.2005.04.055

Danila Merino, Yamila Mansilla, Claudia Casalongué, & Vera Alvarez. (2016). Propiedades Fisicoquimicas Y Antibacteriales De Mezclas Pla-Quitosano Obtenidas Por Casting Con Potencial Uso Como Acolchados Agrícolas. *Avances En Ciencias e Ingeniería*, *7*(1), 27–39.

Dengate, S., & Ruben, A. (2002). Controlled trial of cumulative behavioural effects of a common bread preservative. *J Paediatr Child Health*, *38*, 373–376. https://doi.org/https://doi.org/10.1046/j.1440-1754.2002.00009.x

Dhall, R. K., & Alam, M. S. (2020). Biodegradable Packaging. *Encyclopedia of Renewable and Sustainable Materials*, 26–43. https://doi.org/10.1016/b978-0-12-803581-8.11516-4

dos Santos, J. L. P., Bernardi, A. O., Pozza Morassi, L. L., Silva, B. S., Copetti, M. V., & S. Sant'Ana, A. (2016). Incidence, populations and diversity of fungi from raw materials, final products and air of processing environment of multigrain whole meal bread. *Food Research International*, *87*, 103–108. https://doi.org/10.1016/j.foodres.2016.07.002

Elsabee, M. Z., & Abdou, E. S. (2013). Chitosan based edible films and coatings: A review. *Mat Sci Eng C-Mater*, *33*, 1819–1841. https://doi.org/https://doi.org/10.1016/j.msec.2013.01.010

FAO/WHO. (1995). General Standard for Food Additives. *Codex Alimentarius: International Food Standards*, *192*, 1–475. Retrieved from http://www.fao.org/gsfaonline/docs/CXS_192e.pdf

Ganji, F., & Abdekhodaie, M. J. (2008). Synthesis and characterization of a new thermosensitive chitosan–PEG diblock copolymer. *Carbohyd Polym*, *74*, 435–441. https://doi.org/https://doi.org/10.1016/j.carbpol.2008.03.017

Garcia, M. V., Bernardi, A. O., & Copetti, M. V. (2019). The fungal problem in bread production: insights of causes, consequences, and control methods. *Current Opinion in Food Science*, *29*, 1–6. https://doi.org/10.1016/j.cofs.2019.06.010

Guinault, A., Sollogoub, C., Domenek, S., Grandmontagne, A., & Ducruet, V. (2010). Influence of crystallinity on gas barrier and mechanical properties of pla food packaging films. *International Journal of Material Forming*, *3*(SUPPL. 1), 603–606. https://doi.org/10.1007/s12289-010-0842-9

Hassouna, F., Raquez, J.-M., Addiego, F., Dubois, P., Toniazzo, V., & Ruch, D. (2011). New approach on the development of plasticized polylactide (PLA): Grafting of poly(ethylene glycol) (PEG) via reactive extrusion. *Eur Polym J*, *47*, 2134–2144. https://doi.org/https://doi.org/10.1016/j.eurpolymj.2011.08.001

Heras-Mozos, R., Muriel-Galet, V., López-Carballo, G., Catalá, R., Hernández-Muñoz, P., & Gavara, R. (2019). Development and optimization of antifungal packaging for sliced pan loaf based on garlic as active agent and bread aroma as aroma corrector. *International Journal of Food Microbiology*, *290*(September 2018), 42–48. https://doi.org/10.1016/j.ijfoodmicro.2018.09.024

Hsieh, D. P. H. ., Huang, H.-Y. ., Ling, M.-P. ., Chen, Y.-S. ., Huang, L.-L. I. ., Wu, C.-H. ., & Chiang, C.-F. (2012). Total dietary studies and food safety assessment in Taiwan-food preservatives as an illustration. *Journal of Food and Drug Analysis*, *20*(4), 744–763.

Jamshidian, M., Tehrany, E. A., & Desobry, S. (2013). Antioxidants Release from Solvent-Cast PLA Film: Investigation of PLA Antioxidant-Active Packaging. *Food and Bioprocess Technology*, *6*(6), 1450–1463. https://doi.org/10.1007/s11947-012-0830-9

Kechichian, V., Ditchfield, C., Veiga-Santos, P., & Tadini, C. C. (2010). Natural antimicrobial ingredients incorporated in biodegradable films based on cassava starch. *LWT - Food Sci Technol*, *43*, 1088–1094. https://doi.org/10.1016/j.lwt.2010.02.014

León, A. E., Durán, E., & de Barber, C. B. (2002). Utilization of Enzyme Mixtures To Retard Bread Crumb Firming. *J Agric Food Chem*, *50*, 1416–1419. https://doi.org/https://dx.doi.org/10.1021/jf0106446

Licciardello, F., Cipri, L., & Muratore, G. (2014). Influence of packaging on the quality maintenance of industrial bread by comparative shelf life testing. *Food Packaging and Shelf Life*, *1*, 19–24. https://doi.org/http://dx.doi.org/10.1016/j.fpsl.2013.10.001

Ling, M. P., Lien, K. W., Wu, C. H., Ni, S. P., Huang, H. Y., & Hsieh, D. P. H. (2015). Dietary exposure estimates for the food preservatives benzoic acid and sorbic acid in the total diet in taiwan. In *Journal of Agricultural and Food Chemistry* (Vol. 63). https://doi.org/10.1021/jf503987y

Ma, P., Hristova-Bogaerds, D. G., Goossens, J. G. P., Spoelstra, A. B., Zhang, Y., & Lemstra, P. J. (2012). Toughening of poly(lactic acid) by ethylene-co-vinyl acetate copolymer with different vinyl acetate contents. *Eur Polym J*, *48*, 146–154. https://doi.org/https://doi.org/10.1016/j.eurpolymj.2011.10.015

Miś, A., Krekora, M., Niewiadomski, Z., Dziki, D., & Nawrocka, A. (2020). Water redistribution between model bread dough components during mixing. *Journal of Cereal Science*, *95*, 103035. https://doi.org/10.1016/j.jcs.2020.103035

Moura, I., Nogueira, R., Bounor-Legare, V., & Machado, A. V. (2012). Synthesis of EVA-g-PLA copolymers using transesterification reactions. *Mater Chem Phys*, *134*, 103–110. https://doi.org/https://doi.org/10.1016/j.matchemphys.2012.02.036

Muzzarelli, R. A. A., Muzzarelli, C., Tarsi, R., Miliani, M., Gabbanelli, F., & Cartolari, M. (2001). Fungistatic Activity of Modified Chitosans against Saprolegnia parasitica. *Biomacromolecules*, *2*, 165–169. https://doi.org/10.1021/bm000091s

Pena, L., & Burton, B. K. (2012). Survey of Health Status and Complications Among Propionic Acidemia Patients. *Am J Med Genet Part A*, *158A*, 1641–1646. https://doi.org/https://doi.org/10.1002/ajmg.a.35387

Phechkrajang, C. M., & Yooyong, S. (2017). Fast and simple method for semiquantitative determination of calcium propionate in bread samples. *J Food Drug Anal*, *25*, 254–259. https://doi.org/https://doi.org/10.1016/j.jfda.2016.03.013

Pinilla, C. M. B., Thys, R. C. S., & Brandelli, A. (2019). Antifungal properties of phosphatidylcholine-oleic acid liposomes encapsulating garlic against environmental fungal in wheat bread. *International Journal of Food Microbiology*, *293*(January), 72–78. https://doi.org/10.1016/j.ijfoodmicro.2019.01.006

Rhim, J. W., Hong, S. I., Park, H. M., & Ng, P. K. W. (2006). Preparation and characterization of chitosan-based nanocomposite films with antimicrobial activity. *Journal of Agricultural and Food Chemistry*, *54*(16), 5814–5822. https://doi.org/10.1021/jf060658h

Sébastien, F., Stéphane, G., Copinet, A., & Coma, V. (2006). Novel biodegradable films made from chitosan and poly(lactic acid) with antifungal properties against mycotoxinogen strains. *Carbohydrate Polymers*, *65*(2), 185–193. https://doi.org/10.1016/j.carbpol.2006.01.006

Sengupta, S., Manna, S., Roy, U., & Das, P. (2020). Manufacturing of Biodegradable Poly Lactic Acid (PLA): Green Alternatives to Petroleum Derived Plastics. In *Encyclopedia of Renewable and Sustainable Materials*. https://doi.org/10.1016/b978-0-12-803581-8.11013-6

Shuaib, T., Al-Hashmi, N., Ghaziuddin, M., Megdad, E., Abebe, D., Al-Saif, A., & Al-Owain, M. (2012). Propionic Acidemia Associated With Visual Hallucinations. *J Child Neurol*, *27*, 799–803. https://doi.org/https://doi.org/10.1177/0883073811426929

Sopiwnyk, E., Young, G., Frohlich, P., Borsuk, Y., Lagassé, S., Boyd, L., & Malcolmson, L. (2020). Effect of pulse flour storage on flour and bread baking properties. *LWT*, *121*, 108971. https://doi.org/https://doi.org/10.1016/j.lwt.2019.108971

Srisa, A., & Harnkarnsujarit, N. (2020). Antifungal films from trans-cinnamaldehyde incorporated poly(lactic acid) and poly(butylene adipate-co-terephthalate) for bread packaging. *Food Chemistry*, *333*(July), 127537. https://doi.org/10.1016/j.foodchem.2020.127537

Sungsanit, K., Kao, N., Bhattacharya, S., & Pivsaart, S. (2010). Physical and rheological properties of plasticized linear and branched PLA. *Korea-Aust Rheol J*, *22*, 187–195.

Suwanamornlert, P., Kerddonfag, N., Sane, A., Chinsirikul, W., Zhou, W., & Chonhenchob, V. (2020). Poly(lactic acid)/poly(butylene-succinate-co-adipate) (PLA/PBSA) blend films containing thymol as alternative to synthetic preservatives for active packaging of bread. *Food Packaging and Shelf Life*, *25*(April), 100515. https://doi.org/10.1016/j.fpsl.2020.100515

Swain, A., Soutter, V., Loblay, R., & Truswell, A. S. (1985). Salicylates, oligoantigenic diets, and behavior. *Lancet*, *326*, 41–42. https://doi.org/https://doi.org/10.1016/S0140-6736(85)90089-3

Tanase, C. E., & Spiridon, I. (2014). PLA/chitosan/keratin composites for biomedical applications. *Materials Science and Engineering C*, *40*, 242–247. https://doi.org/10.1016/j.msec.2014.03.054

Thomas, L. V, & Delves-Broughton, J. (2014). Preservatives: Permitted Preservatives – Sorbic Acid. In C. A. Batt & M. Lou Tortorello (Eds.), *Encyclopedia of Food Microbiology* (2nd ed., pp. 102–107). London, UK: Elsevier Ltd. All.

Torres-Huerta, A. M., Palma-Ramírez, D., Domínguez-Crespo, M. A., Del Angel-López, D., & De La Fuente, D. (2014). Comparative assessment of miscibility and degradability on PET/PLA and PET/chitosan blends. *Eur Polym J*, *61*, 285–299. https://doi.org/http://dx.doi.org/10.1016/j.eurpolymj.2014.10.016

Uz, M., & Altınkaya, S. A. (2011). Development of mono and multilayer antimicrobial food packaging materials for controlled release of potassium sorbate. *Lebensm-Wiss Technol*, *44*, 2302–2309. https://doi.org/https://doi.org/10.1016/j.lwt.2011.05.003

van den Broek, L. A. M., Knoop, R. J. I., Kappen, F. H. J., & Boeriu, C. G. (2015). Chitosan films and blends for packaging material. *Carbohyd Polym*, *116*, 237–242. https://doi.org/10.1016/j.carbpol.2014.07.039

Wang, H., Qian, J., & Ding, F. (2018). Emerging Chitosan-Based Films for Food Packaging Applications. *Journal of Agricultural and Food Chemistry*, *66*(2), 395–413. https://doi.org/10.1021/acs.jafc.7b04528

Yuniarto, K., Purwanto, Y. A., Purwanto, S., Welt, B. A., Purwadaria, H. K., & Sunarti, T. C. (2016). Infrared and Raman studies on polylactide acid and polyethylene glycol-400 blend. *AIP Conference Proceedings*, *1725*. https://doi.org/10.1063/1.4945555

Zhang, H., Li, R., & Liu, W. (2011). Effects of Chitin and Its Derivative Chitosan on Postharvest Decay of Fruits : A Review. *Int J Mol Sci*, *12*, 917–934. https://doi.org/https://doi.org/10.3390/ijms12020917

Zhao, Y.-Q., Cheung, H.-Y., Lau, K.-T., Xu, C.-L., Zhao, D.-D., & Li, H.-L. (2010). Silkworm silk/poly(lactic acid) biocomposites: Dynamic mechanical, thermal and biodegradable properties. *Polym Degrad Stabil*, *95*, 1978–1987. https://doi.org/https://doi.org/10.1016/j.polymdegradstab.2010.07.015