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Potential of extrusion-blown poly(lactic acid)/cellulose nanocrystals nanocomposite films for improving the shelf-life of a dry food product

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ABSTRACT

The potential of neat poly(lactic acid) (PLA) and PLA/cellulose nanocrystal (CNC) blown films in extending the shelf-life of crackers, a moisture-sensitive food product, was assessed. Product's shelf-life, i.e., the time required by the crackers to reach its critical moisture content (CMC) of 8%, was determined experimentally from moisture content versus storage time plots at a constant temperature (25 °C) and various conditions of relative humidity (RH). Neither crackers within PLA nor PLA/CNC packages reached the CMC when stored below 50% RH at 25 °C, indicating that crackers packaged in both films were shelf stable at these conditions. Above this RH, the crackers inside both packages reached the CMC at different times, however crackers in CNC-based packages had approximately 40% longer shelf-life than those in neat PLA package. The shelf-life of crackers packaged in films predicted from various simulation mathematical models supported the experimental data that the crackers packaged in the PLA/CNC films had higher shelf-life compared to those packaged in neat PLA films; clearly demonstrating the potential of CNC-based films to extend the shelf-life of dry-foods.

1. Introduction

The large amount of waste generated from petroleum-based plastics remains a growing environmental concern for the plastic industry (Muller, González-Martínez, & Chiralt, 2017; Parker, 2018). Since consumers are becoming more interested in the environment, their preferences are driving the industry to seek more sustainable alternative materials that are compostable and biodegradable (Muller et al., 2017). Poly(lactic acid) (PLA) is one such bio-based plastic derived from renewable natural resources that has become an attractive alternative because of its sustainable nature, biodegradability, compostability, and other attributes such as good processability, decent mechanical properties, and excellent flavor and aroma barrier (Karkhanis & Matuana, 2019; Karkhanis, Stark, Sabo, & Matuana, 2017). Regardless of these advantages, PLA is fragile and has higher moisture and oxygen permeabilities compared to common petroleum plastics, making it unsuitable for applications such as food packaging where film flexibility and long-term food storage are critical (Matuana, Karkhanis, Stark, & Sabo, 2016; Vijayarajan, Selke, & Matuana, 2014; Xing & Matuana, 2016).

Moisture gain or loss in food products can cause defects such as freezer burn, non-enzymatic browning, starch retrogradation, mold and microbial growth, as well as flavor, color and textural changes, amongst others (Roudaut & Debeaufort, 2010; Siracusa, 2012). For instance, dry foods (e.g., crackers) and moist products (e.g., cake) are often susceptible to textural changes such as sogginess and brittleness due to moisture gain and loss, respectively, so they require a package that can provide some moisture barrier. Oxygen-sensitive food products are susceptible to oxidative reactions resulting in loss of nutrients and other undesirable changes in flavor, appearance as well as texture (Roudaut & Debeaufort, 2010; Siracusa, 2012). These products need packages that provide some oxygen barrier. Improving barrier properties prolongs product shelf-life, defined as a time that foods are given before they are considered unsuitable for use or consumption. Longer shelf-lives impact both food safety and waste avoidance.

Recently, cellulose nanomaterials have been considered for sustainable packaging applications as barrier films (Wang et al., 2018). While conventional macroscopic cellulose has generally been considered safe for food packaging applications, some concerns regarding the migration of nanocellulose into food and its toxicity on human health have been expressed. Anticipating cellulose nanomaterials as a potential food additive or for use in sustainable food packaging, several researchers addressed the potential cytotoxicity, genotoxicity and ecotoxicity of

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these nanomaterials, amongst others (Ede et al., 2020; Fotie, Limbo, & Piergiovanni, 2020; Ong et al., 2020; Pradhan et al., 2020). Remarkably, no or very low cytotoxic, genotoxic and ecotoxic effects of CNCs have been reported through several in vitro and in vivo analysis, leading to its regulatory approval in several countries such as Canada (Fotie et al., 2020). Indeed, food safety studies commissioned by a consortium of 10 North American organizations partnering with academic, governmental, and commercial labs assessed the safe use of fibrillated cellulose and nanocrystalline cellulose in food (Ede et al., 2020; Ong et al., 2020; Pradhan et al., 2020). In vivo and in vitro results published in 2020 by this consortium demonstrate that both forms of cellulose are as safe to use in food up to 4 wt% as conventional cellulose. Other investigators assessed the effect of CNCs on the migration properties of PLA nano-bio composites. Interestingly, the overall migration levels in both polar and nonpolar food simulants were found well below the standard limits needed for food packaging materials (Fortunati et al., 2012; Silva, Dourado, Gama, & Pocas, 2020). The low migration rates from packages into food combined with no demonstrated toxicity in food up to 4% clearly show the potential of CNC-based films for food contact applications.

Our previous studies demonstrated that the poor moisture and moderate gas barrier properties of PLA are significantly improved by incorporating various amounts of cellulose nanocrystals (CNCs) (0-2 wt %) into the films (Karkhanis, Stark, Sabo, & Matuana, 2017; Karkhanis, Stark, Sabo, & Matuana, 2018). When CNC was added into PLA matrix, the moisture and oxygen barrier properties of the resulting nanocomposite films improved in the range of 33-50% and 62-70%, respectively, compared to the films made from PLA alone (Karkhanis et al., 2017b, 2018). This significant improvement occurred at an optimum CNC content of 1% and was attributed to the increased crystallinity and tortuosity effect induced by the CNC addition. Extensive work has been previously done to characterize PLA films containing various amounts of CNC in terms of CNC dispersion and distribution (Karkhanis et al., 2017b), crystallinity and molecular weight (Karkhanis et al., 2017b, 2018), and water and oxygen barrier at various relative humidities and temperatures (Karkhanis et al., 2018).

Despite the barrier attributes of PLA/CNC films, it is unknown how they will impact the shelf-life of moisture and/or oxygen-sensitive food products. This study evaluated the potential of PLA/CNC films in extending the shelf-life of crackers, which are moisture-sensitive products. Shelf-life determination of food product-package systems using experimental techniques can be very time and labor intensive, making the shelf-life prediction using mathematical models very crucial for making commercial decisions within tight time constraints (Rachtanapun, 2007). Thus, in this study the shelf-life of crackers packaged in films was also predicted from various simulation mathematical models and compared to the experimental data.

2. Material and methods

2.1. Materials

Saltine crackers (2 inches by 2 inches), moisture-sensitive products, were purchased in a local supermarket in Lansing, Michigan (Meijer Select saltine crackers brand) with fat, protein, and total carbohydrate contents of 10.71%, 7.14%, and 71.43%, respectively. Salts such as lithium chloride, magnesium chloride, magnesium nitrate, sodium nitrite, ammonium chloride and potassium nitrate (ACS grade) were used to create saturated salt solutions (Anstice, Page, & Page, 2005; Arabhosseini, Huisman, van Boxtel, & Muller, 2005; Young, 1967). Ingeo™ biopolymer 4044D, a reactive extrusion grade of PLA from NatureWorks® LLC (Minnetonka, MN) was used in pellet form. This grade was selected due to its film forming ability and reactivity which allows for chain branching if needed. Its density provided by the manufacturer was 1.24 g/cm^3. Whereas its melt index (190 °C, 2.16 kg), number and weight average molecular weights were 11.5 g/10 min, 123-142 kDa, and 212-272 kDa, respectively (Karkhanis & Matuana, 2019; Karkhanis et al., 2017b). Cellulose nanocrystals (Batch # 2012-FPL-CNC-043) were produced by dissolving wood pulp using the sulfuric acid process at the Forest Products Laboratory in Madison, Wisconsin, USA (Moon, Beck, & Rudie, 2013). The sulfur content of the CNCs was about 1 wt% (Dunlop et al., 2020). Electron micrographs of CNCs prepared in the FPL pilot plant can be found elsewhere (Postek et al., 2011). The lengths of these CNCs as-prepared varies but are typically around 100 nm, and the diameters are typically 5-10 nm with typical aspect ratios of about 10-20 (Postek et al., 2011). Freeze-dried CNCs typically appear as flakes that are 10-100 micrometres in size, and micrographs of these freeze-dried CNCs can be found elsewhere (Stark, Wei, Sabo, Reiner, & Matuana, 2018).

2.2. Packaging materials (PLA and PLA/CNC films)

2.2.1. Film manufacturing

Oven-dried PLA pellets (50 °C for at least a day) were mixed with 1 wt% CNC in a high intensity blender for approximately 1 minute, and the blended mixture was blown into ~0.027 mm thick film. Blown film process was carried out in a 19 mm single screw extruder with L:D ratio of 30:1 (C.W. Brabender Instruments, South Hackensack, NJ, USA) at 35 rpm and a constant processing temperature of 180 °C in all four heating zones as previously described (Karkhanis et al., 2017b). An internal air pressure of 0.517 kPa (0.075 psi) and take-up roller speed of 40 rpm were used. Neat PLA film was also manufactured as a control using similar processing conditions. Although PLA film is challenging to manufacture and can be brittle compared to polyolefin counterparts, in this study it was still flexible enough to allow all property characterization. An illustration of extrusion blown PLA film used in this study can be seen in our previous paper (Karkhanis et al., 2017a).

2.2.2. Water vapor permeability

Water vapor permeability (P) was calculated by the following equation (Karkhanis et al., 2017b, 2018; Matuana et al., 2016):

$$P = \frac{WVTR \times l}{\Delta p} \tag{1}$$

with l as the thickness of the film; Δp the applied pressure difference; and WVTR the water vapor transmission rate of the films measured using 31.67×10^{-4} m^2 as the effective area of exposed film. WVTR was measured gravimetrically in five replicates after 24 hours at 23 °C and 50% to 85% relative humidity (RH) according to the desiccant method procedure described in ASTM standard E 96 (Karkhanis et al., 2017b, 2018).

2.3. Moisture sorption isotherm of crackers

2.3.1. Initial moisture content (IMC)

Six aluminum dishes were weighed using the Ohaus Discovery Analytical Balance (Ohaus Corp., Parsippany, NJ) without the product (crackers). The saltine crackers were crushed, and 2-3 grams were placed into the aluminum dishes and weighed again. The aluminum dishes with the crackers were then placed into an oven for drying at 100 ± 2 °C until a constant weight was achieved. The dishes were re-weighed to determine the weight loss of the product. The initial moisture content (IMC) on dry basis was calculated using the following equation (Azanha & Faria, 2005; Rachtanapun, 2007; Sirpatrawan, 2009):

$$IMC\ (\%) = \frac{W_i - W_D}{W_D} \times 100 \tag{2}$$

where W_i is the initial weight of the product (before drying) (in g) and W_D is the weight of the product after drying (in g) in the oven.

2.3.2. Equilibrium moisture content (EMC)

Six different saturated salt solutions were used in desiccators to provide constant relative humidity (RH) conditions ranging from 10% to 96% (Table 1). These desiccators were further placed in an oven set to 25 °C to maintain a constant temperature. Six samples of approximately 2-3 grams of saltine crackers were put in aluminum dishes and placed in each of the six desiccators containing different saturated salt solutions (various RH conditions). The samples were allowed to equilibrate for a period of at least two weeks until there was no discernable weight gain. The EMC of the samples was then calculated using the following equation (Azanha & Faria, 2005; Rachtanapun, 2007; Sirpatrawan, 2009):

$$EMC\ (\%) = \left[\frac{W_e}{W_i}(IMC + 1) - 1\right] \times 100 \quad (3)$$

where W_e is the weight of crackers at equilibrium (g), W_i is the initial weight of crackers (g) and IMC is the initial moisture content calculated from the previous Section 2.3.1.

The moisture sorption isotherm was constructed as a plot of the EMC values of the crackers as a function of their storage RH (Azanha & Faria, 2005; Rachtanapun, 2007; Sirpatrawan, 2009).

The experimental EMC values were compared to those predicted by the Guggenheim Anderson de Boer (GAB) equation (Azanha & Faria, 2005; Rachtanapun, 2007; Sirpatrawan, 2009):

$$EMC_{pred} = \frac{C \cdot K \cdot a_w \cdot W_m}{(1 - K \cdot a_w) \cdot (1 - K \cdot a_w + C \cdot K \cdot a_w)} \quad (4)$$

where EMC_{pred} is the predicted equilibrium moisture content of the product, C is the Guggenheim constant, K is the correction factor, W_m is the monolayer saturated water content, and a_w is the water activity (given as RH/100).

In order to calculate the C, K and W_m values in equation (4), a plot of the experimental values for a_w/EMC as a function of a_w was generated. A quadratic trend was then fitted to the plot using the following equation (Azanha & Faria, 2005; Andrade, Lemus, & Perez, 2011; Rachtanapun, 2007; Robertson & Lee, 2021; Sirpatrawan, 2009; van den Berg, 1985):

$$\frac{a_w}{EMC} = \alpha \cdot \left(a_w^2\right) + \beta \cdot a_w + \gamma \quad (5)$$

where α, β and γ are constants of the quadratic equation used to fit the experimental data.

Once the values of α, β and γ are known, the following equation can be used to estimate the values of C (Robertson & Lee, 2021; Kilcast & Subramaniam, 2011):

$$C = \frac{T + \sqrt{T^2 - 4T}}{2} \quad (6)$$

Where T is given by:

$$T = \frac{\beta^2}{-\alpha \cdot \gamma} + 4 \quad (7)$$

The values of K and W_m were obtained by solving the following equations simultaneously (Robertson & Lee, 2021; Kilcast & Subramaniam, 2011):

$$\alpha = \frac{K}{W_m}\left[\frac{1}{C} - 1\right] \quad (8)$$

$$\gamma = \frac{1}{W_m \cdot C \cdot K} \quad (9)$$

The values of the GAB constants (C, K and W_m) obtained experimentally are listed in Table 2 and can be used to predict the EMC values of the crackers at any relative humidity condition from equation (4).

Generally, the root mean square (RMS) is determined to assess the precision of fit of the GAB model to the experimental data as follows (Azanha & Faria, 2005; Rachtanapun, 2007; Sirpatrawan, 2009):

$$RMS = \left[\sqrt{\frac{\sum_{i=1}^{N}\left(\frac{EMC_{exp} - EMC_{pred}}{EMC_{pred}}\right)^2}{N}}\right] \times 100 \quad (10)$$

where EMC_{exp} is the experimental equilibrium moisture content, EMC_{pred} is the equilibrium moisture content predicted by the GAB model, and N is the number of experimental points. Generally, a low RMS value (below 10%) signifies a good fit of the model to the experimental data.

Table 1
Theoretical and measured relative humidity (% RH) for various saturated salt solutions at 25 °C.

Salt	% RH for saturated salt solutions at 25°C	
	Theoretical	Measured
Lithium chloride	11.3	10.4 ± 0.8
Magnesium chloride	32.8	31.8 ± 1.8
Magnesium nitrate	52	50.1 ± 3.5
Sodium nitrite	65	61.1 ± 3.5
Ammonium chloride	78.8	78.5 ± 5.3
Potassium nitrate	93.6	96.1 ± 5.9

2.4. Shelf-life determination

2.4.1. Shelf-life of packaged crackers

Shelf-life of packaged crackers was measured using vapometer cup assemblies (EZ-Cup Vapometer, Thwing-Albert Instrument Company). The cup was closed by placing film samples (PLA and PLA/CNC) between a lightweight aluminum cup and an aluminum threaded flanged ring with two neoprene gaskets with a Teflon seal to hold the films in place. The vapometer cup assemblies contained approximately 10 grams of crushed crackers and were placed into the desiccators set at various RH conditions. The desiccators were then placed in an oven set at 25 °C to maintain a constant temperature. The vapometer cup assemblies were weighed periodically over a one-month period to determine the percent moisture gain until the equilibrium moisture content (EMC) was reached (steady state).

From the data, plots of moisture content versus storage time were generated. The product's shelf-life, i.e., the time required by the crackers to reach its critical moisture content (CMC), was determined from moisture content versus storage time plots. Generally, CMC is determined according to the sensory evaluation as the minimum moisture content at which the product becomes organoleptically unacceptable, for example losing its crispiness. Critical moisture content values of 6.1% for wheat crackers (Hao, Lu, & Wang, 2016); 6.8% for rice crackers (21.09% fat, 6.31% protein, 68.39% total carbohydrate) (Sirpatrawan, 2009); as well as 10% and 8% for lean and rich crackers, respectively (Amemiya & Menjivar, 1992; Kaletunc & Breslauer, 2003) have been reported in the literature based on the sensory evaluation for crackers; rice crackers; and crackers, respectively. Nevertheless, a CMC value of 8% was adopted in this study, an average between the CMC values of 6% and 10% reported in the literature for wheat crackers (Amemiya & Menjivar, 1992; Hao et al., 2016; Kaletunc & Breslauer, 2003; Sirpatrawan, 2009).

Table 2
Values of GAB constants.

GAB Constants	Values
C	19.35
K	0.78
W_m (g/g dry basis)	0.06

2.4.2. Shelf-life modeling

Several mathematical models for predicting the shelf-life of a moisture sensitive product packaged in a film have been described in literature. Some of these models, such as the linear, middle-point, logarithmic, and GAB model have been extensively described and used to predict the shelf-life of crackers in PLA and PLA/CNC films in this paper. Table 3 lists these models and their respective predictive shelf-life equations for packaged moisture-sensitive products (Azanha & Faria, 2005; Rachtanapun, 2007; Robertson & Lee, 2021; Sirpatrawan, 2009). The linear shelf-life prediction model is the simplest one. In this model, the sorption isotherm data is modelled linearly in the range of interest (between IMC and CMC). In the middle point method and logarithmic methods, the region of interest is divided into "n" intervals and the value of time needed to change the product's moisture content between the limits of each interval is calculated. The larger the number of intervals (minimum of three), the smaller the error (Azanha & Faria, 2005; Kilcast & Subramaniam, 2011; Rachtanapun, 2007; Robertson & Lee, 2021; Sirpatrawan, 2009). All of the above models have been used to predict the shelf-life of moisture sensitive food products (Azanha & Faria, 2005; Kilcast & Subramaniam, 2011; Rachtanapun, 2007; Robertson & Lee, 2021; Sirpatrawan, 2009).

Although the models mentioned previously have been used to predict the shelf-life of dry foods packaged in films, the GAB equation is the most commonly used model. The shelf-life of a food-package system predicted by the GAB equation can be determined as follows (Robertson & Lee, 2021):

$$t = \frac{H}{\varepsilon \cdot \varnothing} \tag{11}$$

with H, ε and ∅ are given by:

$$H = M_f - M_i + \frac{2 \cdot W_m \cdot C}{\varepsilon} \ln\left(\frac{\varepsilon \cdot M_f - 2 \cdot W_m \cdot C}{\varepsilon \cdot M_i - 2 \cdot W_m \cdot C}\right) \tag{12}$$

$$\varepsilon = (1 - C)(2 \cdot K \cdot a_{wo} - 2) \tag{13}$$

$$\varnothing = \frac{A \cdot P \cdot P_s}{2 \cdot K \cdot (1 - C) \cdot l \cdot W_D} \tag{14}$$

where C, W_m and K are GAB constants (listed in Table 2), M_f and M_i are the critical and initial moisture contents of the food, a_{wo} is the water activity at the storage condition (RH/100), A is area of the package, P is the permeability of the packaging material, P_s is the saturated vapor pressure at food storage temperature, l is the thickness of the packaging material; W_D is the weight of product in the package.

Table 3
Models to predict shelf-life (t) of moisture sensitive foods.

Models	Equations
Linear	$t = \frac{l \cdot W_D \cdot \beta}{P \cdot A \cdot P_s} \ln\left(\frac{a_{wo} - a_{w,t=0}}{a_{wo} - a_{w,t=t}}\right)$
Middle-point	$t = \frac{l \cdot W_D}{P \cdot A \cdot P_s} \sum_{i=1}^{n} \frac{\Delta M_i}{a_{wo} - \left(\frac{a_{w,t=i} + a_{w,t=i+1}}{2}\right)}$
Logarithmic	$t = \frac{l \cdot W_D}{P \cdot A \cdot P_s} \sum_{i=1}^{n} \beta_i \ln\left(\frac{a_{wo} - a_{w,t=i}}{a_{wo} - a_{w,t=i+1}}\right)$

where l is the thickness of the packaging material; W_D is the weight of product in the package; P is the permeability of the packaging material; A is area of the package; P_s is the saturated vapor pressure at food storage temperature; a_{wo} is the water activity at the storage condition (RH/100); β is the slope of the straight line; $a_{w,t=0}$ is the water activity at initial moisture content; $a_{w,t=t}$ is the water activity at critical moisture content; ΔM_i is moisture content change in each interval "i"; $a_{w,t=i}$ and $a_{w,t=i+1}$ are the water activities at the beginning and end of each interval "i", respectively; and β_i is the slope of the line at each interval "i".

3. Results and discussion

3.1. Moisture sorption of food

The initial moisture content (IMC) of the crackers was 4.1% (dry basis), in corroboration with other researchers (Sirpatrawan, 2009). Since the IMC is low, the product has a tendency to absorb moisture from its surrounding atmosphere, making it a moisture-sensitive product. On absorbing moisture, the product becomes soggy and unappetizing. It is important to establish the sorption behavior of food products to estimate product stability at various storage conditions and evaluate the requirements of packaging materials in order to ensure adequate product shelf-life. Therefore, the moisture content of unpackaged crackers was monitored under various conditions of relative humidity (RH) as a function of time (Fig. 1). Two distinct trends were seen from Fig. 1. Firstly, the moisture content of crackers increased as the RH increased, irrespective of the storage time. As expected, the moisture sorption of food increased with RH since more water molecules were available in the atmosphere to be absorbed at a higher RH, leading to a higher concentration gradient, as per Fick's law. Secondly, the moisture content of food increased gradually as the storage time increased and then levelled off for each RH condition, indicating that the product is susceptible to moisture gain due to its low IMC. The moisture content at the equilibrium condition, known as the equilibrium moisture content (EMC), was plotted against RH to generate the moisture sorption isotherm.

Fig. 2 shows a plot of the experimental EMC of the food (obtained from Fig. 1) as a function of the storage RH at 25 °C. The obtained experimental moisture isotherm (Fig. 2) exhibited the sigmoidal (Type-II) or S-shape curve typically observed by other researchers in moisture-sensitive foods such as salted crackers (Rachtanapun, 2007), rice crackers (Sirpatrawan, 2009) and cornflakes (Azanha & Faria, 2005). The EMC increased gradually at lower RH but rapidly at higher RH.

The experimental moisture sorption data was fitted using non-linear regression analysis to the Guggenheim Anderson de Boer (GAB) from equation (4) (Fig. 2) with the RMS value of 3.8% indicating that the GAB model was a good fit to the experimental data obtained for crackers. Our results corroborate other studies for moisture-sensitive foods such as salted crackers (Rachtanapun, 2007), rice crackers (Sirpatrawan, 2009), and cornflakes (Azanha & Faria, 2005). While several investigators have reported the usefulness of the GAB equation to fit sorption isotherms, it should be noted that this model works best below 90% RH (Azanha & Faria, 2005).

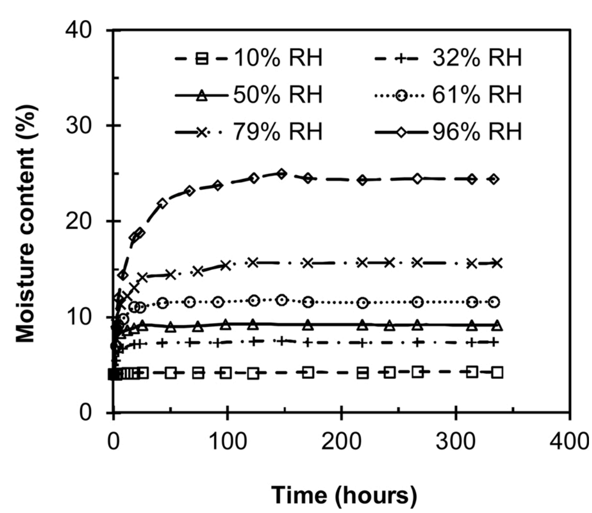

Fig. 1. Moisture contents of crackers as a function of exposure time recorded at 25 °C and various storage relative humidity conditions. The coefficient of variations of the data varied from 0.9 to 3.7%.

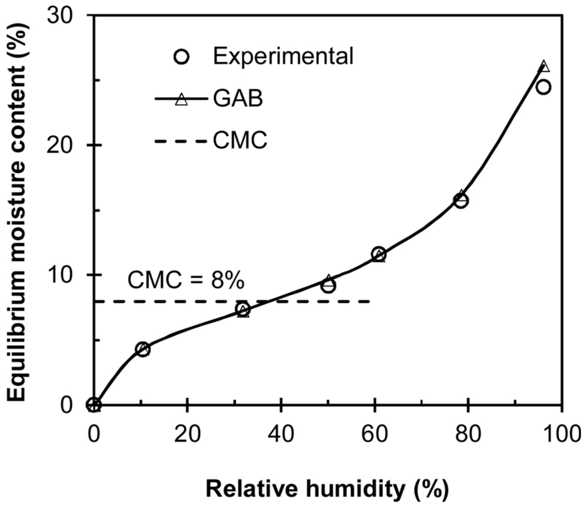

Fig. 2. Moisture sorption isotherm of crackers at 25 °C showing experimental EMC values and those predicted from GAB equation as well as the critical moisture content (CMC). The coefficient of variations of the experimental data varied from 0.4 to 4.4%.

The average critical moisture content (CMC) of 8% intersects the moisture isotherm at 37% RH (Fig. 2). This result implies that the food product is shelf-stable when stored at a RH below 37% since the EMC values obtained below 37% RH are below the CMC. In other terms, when the food product is stored below 37% RH, it will reach an equilibrium moisture content before the critical moisture content, making it shelf-stable below this storage relative humidity. On the other hand, when the food product is stored above 37% RH, it will absorb moisture and eventually reach the CMC, beyond which it will be unacceptable to the consumer. Packaging materials such as PLA and PLA/CNC films can be used to delay this moisture absorption and hence extend the shelf-life of food.

3.2. *Shelf-life of crackers packaged in PLA and PLA/CNC composite films*

Our previous studies demonstrated that the moisture and oxygen barrier properties of PLA films improved in the range of 33-50% and 62-70%, respectively, when only 1% CNC was added into PLA matrix (Karkhanis et al., 2017b, 2018; Matuana et al., 2016). In this work, the potential of these PLA/1%CNC composite films in extending the shelf-life of crackers was assessed.

Table 4 summarizes the shelf-life of crackers packaged in PLA and PLA/CNC films stored at 25 °C and various RH conditions. The crackers packaged in both PLA and PLA/CNC films did not reach the 8% CMC when stored below 50% RH, indicating shelf-stability at these storage conditions. It is worth noting that unpackaged crackers were shelf-stable up to 37% RH only, and that the packaging materials increased the storage relative humidity that the crackers were shelf-stable at 50% RH. The crackers packaged in both PLA and PLA/CNC films reached the 8% CMC when stored above 50% RH. However, the time required to reach the CMC (known as shelf-life) varied with the storage environmental condition (% RH) and the packaging film composition. Firstly, the shelf-life of crackers packaged decreased with increasing RH, irrespective of the film composition. This trend is due to the higher number of water molecules present in the atmosphere at high RH, resulting in high moisture gradient between the inside of the package and the storage environment. As moisture gradient increased, the water vapor transmission rates increased leading to reduced shelf-life, irrespective of the packaging material composition. Secondly, above 50% RH, the shelf-life of crackers packaged in PLA/CNC films was longer than those packaged in unfilled PLA films, irrespective of the storage RH (Table 4). This is attributable to the superior water barrier performance of the PLA/CNC films compared to the neat PLA counterpart films (Table 5). Remarkably, above 50% RH, the shelf-life of crackers packaged in the PLA/CNC films was approximately 40% longer than the crackers packaged in neat PLA films, irrespective of the storage RH, in corroboration with the improved water barrier performance of the nanocomposite films (~40%).

Table 4
Time for crackers packaged in PLA and PLA/CNC films to reach critical moisture content of 8% at different relative humidity conditions and a constant temperature of 25 °C.

% RH	Shelf-life (hours)		
	PLA[1]	PLA/1% CNC[1]	Delayed time (hours) to reach CMC
10	Shelf-stable	Shelf-stable	
33	Shelf-stable	Shelf-stable	
50	Shelf-stable	Shelf-stable	
61	63.4 ± 0.6^{A}	86.1 ± 1.6^{B}	22.7
79	42.9 ± 0.7^{A}	59.9 ± 5.1^{B}	17.0
99	14.2 ± 0.2^{A}	20.1 ± 0.4^{B}	5.9

[1] A one-way t-tests with an α of 0.05 was conducted to compare the shelf life for crackers packaged in PLA and PLA/CNC films at a specific % RH and different superscript letters indicate significant difference.

3.3. *Shelf-life modelling of crackers packaged in PLA and PLA/CNC in films*

Shelf-life determination of food product-package systems using experimental techniques can be very time and labour intensive, depending on the type of food product, packaging material and storage conditions. In fact, even slight changes in a product's ingredients and processing, packaging materials, as well as storage environment can drastically change the shelf-life of the system. Since food manufacturers are constantly changing their product-packaging system for superior product performance and cost reduction, shelf-life prediction using mathematical models becomes very crucial for making commercial decisions within tight time constraints. However, it should be noted that all shelf life simulation models are approximations, and therefore have associated error. In most cases, the expected error is 10-50% or higher, based on the assumptions made (Azanha & Faria, 2005; Kilcast & Subramaniam, 2011; Rachtanapun, 2007; Robertson & Lee, 2021; Sirpatrawan, 2009).

The experimental shelf-life and the predicted shelf-life (using the previously described models) of crackers packaged in PLA and PLA/CNC composite films at several storage relative humidity conditions are listed in Table 6. Interestingly, shelf life predictions support the experimental data that the crackers packaged in the PLA/CNC composite films have a longer shelf-life compared to those packaged in PLA films, irrespective of the storage relative humidity and mathematical models employed (Table 6). These results clearly demonstrate the potential of PLA/CNC films to extend dry-foods' shelf-life compared to neat PLA films.

Rachtanapun reported that the predicted food shelf-life should be shorter or closer than the experimental one for consumer safety

Table 5
Water vapor permeability values of PLA and PLA/CNC films at different relative humidity conditions and a constant temperature of 23 °C.

% RH	Water vapor permeability (10^{-16} kg-m/m^{2}-s-Pa)		
	PLA	PLA/1% CNC	% Decrease
50	327.2 ± 16.1	166.9 ± 4.1	49
57	331.7 ± 20.6	182.7 ± 5.0	45
64	330.7 ± 27.3	187.7 ± 2.9	43
71	318.9 ± 21.3	181.6 ± 3.6	43
78	314.8 ± 43.9	196.5 ± 6.1	38
85	341.8 ± 50.0	200.3 ± 10.0	41

Table 6
Predicted and experimental shelf-life of crackers packaged in PLA and PLA/CNC films stored at various relative humidity conditions.

Models	Shelf-life (hours)					
	61% RH		79% RH		96% RH	
	PLA	PLA/CNC	PLA	PLA/CNC	PLA	PLA/CNC
Linear	24.9	43.9	19.2	33.8	13.4	23.6
Middle-point	13.2	23.3	11.3	19.9	8.9	15.7
Logarithmic	21.4	37.7	16.4	29.0	11.5	20.2
GAB	21.7	38.2	16.5	29.1	11.4	20.1
Experimental	63.4	86.1	42.9	59.9	14.2	20.1

(Rachtanapun, 2007), in agreement with our results clearly showing that all shelf-life models predicted values shorter than or equal to the experimental one (Table 6). Nevertheless, the middle point predicted the lowest shelf-life value compared to other predictive models probably due to single interval of water activity used in the prediction in contrast to other models that use more intervals. The lower number of intervals used in the middle-point method results in a larger predictive error (Azanha & Faria, 2005; Kilcast & Subramaniam, 2011; Rachtanapun, 2007; Robertson & Lee, 2021; Sirpatrawan, 2009).

Notice that the shelf-life values predicted by the mathematical models did not fit those obtained experimentally up to 79% RH (Table 6). These differences between the experimental and predicted shelf-life could be due to the limitations of the mathematical models, which predict shelf-life based solely on the relationship between moisture content of the food product and barrier property of the packaging material. Besides the moisture content of food product and the package permeability, other factors such as food structure and composition may also significantly affect the shelf-life characteristics of the packaged foods. For instance, the salt present in the crackers can affect the moisture absorption and hence shelf-life prediction through the modelling techniques used. Other researchers have also reported large deviations in the predicted and experimental shelf-life values of salted crackers (Rachtanapun, 2007) and rice crackers (Sirpatrawan, 2009) attributable to the presence of additional food constituents such as salt (Rachtanapun, 2007) and fat (Sirpatrawan, 2009). Additional factors such as packaging film roughness as well as fluctuations in storage temperature, and relative humidity during testing, amongst others could also affect the accuracy of shelf-life predicted by mathematical models. Indeed, the tested PLA/CNC films had some degree of roughness (Liu & Matuana, 2019), which could have also contributed to experimental errors and inaccurate shelf-life predictions. Packaging films themselves may have absorbed moisture. Surprisingly, all models at 96% RH gave values very close to the experimental ones and this trend cannot be explained now and needs further investigation.

4. Conclusions

Crackers packaged within both PLA and PLA/CNC composite films did not reach the critical moisture content (CMC) of 8% when stored below 50%RH at 25 °C, indicating that the packaged crackers were shelf-stable at these conditions. Above a storage RH of 50%, the crackers inside both packages reached the CMC but at different times with CNC-based packages having approximately 40% longer shelf-life compared to the neat PLA package, irrespective of the RH.

The linear, middle-point, logarithmic and GAB mathematical models were used to estimate the shelf-life of crackers packaged in PLA and PLA/CNC films. However, none of the models could accurately predict the shelf-life of the product-package system attributable to factors such as the food composition, thickness variations in packaging films and fluctuations in storage temperature and relative humidity. Nevertheless, all models predicted a longer shelf-life for crackers packaged in the PLA/CNC composite films compared to their neat PLA counterpart films, irrespective of the relative humidity.

The experimental results clearly demonstrated the potential of PLA/CNC films to extend the shelf-life of dry-foods compared to neat PLA films.

Declaration of Competing Interest

The authors report no declarations of interest.

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