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Tapioca starch active nanocomposite films and their antimicrobial effectiveness on ready-to-eat chicken meat

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ABSTRACT

Tapioca starch active nanocomposite films were prepared by incorporating cellulose nanocrystal and two grape pomace extracts (Cabernet Franc (red variety) and Viognier (white variety) using a solvent casting method. Their physical properties and antibacterial activities were characterized. Incorporating cellulose nanocrystal significantly ($P < 0.05$) increased the films tensile strength and decreased their elongation at break and water vapor permeability. GPE had a compensatory effect on mechanical properties and water vapor permeability. The films incorporating Viognier and the presence of cellulose nanocrystal led to higher amounts of phenolic compound release. The films incorporating grape pomace extracts exhibited a stronger inhibitory effect on *S. aureus* ATCC 29213 compared to *L. monocytogenes* ATCC 7644. Further application of the films on ready-to-eat chicken meats indicated that starch/cellulose nanocrystal/Viognier films exhibited the most effective against *L. monocytogenes* inoculated on the meat samples during the 10 days storage period at 4 °C.

1. Introduction

Food packaging materials manufactured from nonrenewable petroleum resources account for approximately 50% of plastics (100 million tons) used annually on a global basis (Marsh & Bugusu, 2007). In the US, about 6% of plastics used for packaging is recycled or reused and the remaining is buried in landfills or combusted (Marsh & Bugusu, 2007), which raises concerns about environmental impacts and sustainability. Biodegradable food packaging materials from renewable resources offer a promising, sustainable approach to minimize environmental impacts (Sanchez-Garcia, Lopez-Rubio, & Lagaron, 2010).

Of many biodegradable materials, starch is a well-known candidate material for replacement of petroleum-derived synthetic polymers due to its low cost, availability, and total degradability after usage. Among different starch sources, tapioca starch has been shown great potential to make transparent and colorless flexible films without any previous chemical treatment (Maran, Sivakumar, Sridhar, & Thirugnanasambandham, 2013; Vicentini, Dupuy, Leitzelman, Cereda, & Sobral, 2005). However, hydrophilic character and consequently poor mechanical properties of starch film in presence of water and humid environments limits its wide application (Jimenez, Fabra, & Talens, 2012). Of various physical or chemical means used to overcome these shortcomings, development of nanocomposites by incorporating cellulose nanocrystal is a feasible method to improve functional properties of starch film (George & Siddaramaiah, 2012; Kaushik, Singh, & Verma, 2010; Miri et al., 2015). Cellulose nanocrystals (CNC) are the crystalline residue produced after acid hydrolysis of cellulose, which is the most abundant, renewable, and biodegradable resource on earth (Dufresne, 2008). Nano-scale dimensions and high surface area-to-volume ratio of CNC render them the most promising biodegradable nanofiller for reinforcement of a polymer matrix (Chen, Lawton, Thompson, & Liu, 2012).

Consumer demand for safe, minimally processed, and ready-to-eat (RTE) food products poses major challenges and opportunities for the food industry (Jutaporn, Suphitchaya, & Thawien, 2011). In the US, up to 100 million people annually are affected by foodborne-related acute gastroenteritis. Of all microbiologically-related Class I food recalls, especially for RTE foods, about two thirds are attributed to contamination caused by post-processing mishandling and faulty packaging (Gounadaki, Skandamis, Drosinos, & Nychas, 2007). Microbial contamination occurs primarily at the surface for many refrigerated food products (Chen, Ren et al., 2012). Antimicrobial food packaging, one type of active packaging through incorporating antimicrobial agents directly in the packaging matrix, is an innovative and efficient post-processing strategy against contamination and/or minimizing the level of pathogenic microorganisms in food products (Imran et al., 2010). Packaging films function as carriers for antimicrobial agents which could diffuse into contained food to control target microorganisms (Jutaporn et al., 2011).

Public concern over the synthetic food additives makes naturally-

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occurring antimicrobial agents, including essential oils from plants, enzymes from animal sources, bactieriocins from microbial sources and organic acids as a preferred alternative for food preservation (Fortunati et al., 2017; Gyawali & Ibrahim, 2014; Kuorwel, 2011; Lucera, Costa, Conte, & Nobile, 2012). Except those, grape pomace extract (GPE) is also one option because it contains high levels of polyphenols with strong antioxidant and antimicrobial activities (Garcia-Lomillo, Gonzalez-SanJose, Pino-Garcia, Rivero-Perez, & Muniz-Rodriguez, 2014; Xu, Burton, Kim, & Sismour, 2016). Grape pomace refers to the solid remains, consisting of skin, pulp, seeds, and stems following pressing of fruits for juice or winemaking. Large quantities of grape pomace are annually produced, and most of which is discarded (González-Centeno et al., 2013). Although the interest in polyphenols as naturally-occurring antimicrobial agents is increasing, the work on incorporating GPE in packaging film is very limited.

In this regard, we conducted a two-part investigation: (1) preparing and characterizing starch active nanocomposite films incorporating GPE as an antimicrobial agent and CNC as a strengthening agent, and (2) applying the films to RTE chicken meats and evaluating their antimicrobial properties.

2. Materials and methods

2.1. Materials

Tapioca starch was provided by a commercial supplier. CNC paste made from wood pulp was purchased from the Processing Development Center at University of Maine. Grape pomaces of Cabernet Franc (CF, red variety and Viognier (VN, white variety) were obtained from a winery in Goochland County, Virginia, US. The pomaces were hand-sorted to remove debris and stems, then freeze-dried and ground using an IKA micro-mill (Wilmington, NC, US) to pass through a size–20 mesh sieve. Extracts were prepared by mixing 10 g of ground sample with 40 mL of aqueous acetone (80% v/v), followed by stirring for 24 h at room temperature, and then centrifugation at 12,000*g* for 20 min at 4 °C. Supernatant was decanted into pre-weighed dishes and dried in a chemical hood with constant air flow. Dried extracts were weighed, suspended in distilled water, and then filtered through 0.2 μm syringe filters prior to conducting experiments.

2.2. Film preparation

Starch solutions with concentration of 5% (w/v) were prepared by dispersing tapioca starch in distilled water, heating the mixtures on hotplates with stirring until gelatinization, and then cooling to 25 °C. Glycerin was added as 30% (v/w) of the starch weight in the film-forming solutions. CNC paste was added as 10% (v/v) of starch solution, while two GPEs, namely CF and VN, were added as 8% (v/v) of the film-forming solution. The mixtures were homogenized for 2 min using a BioSpec homogenizer (Bartlesville, OK, US) and cast onto flat, level Teflon-coated plates. After drying at room temperature for 72 h, they were carefully peeled from the plates and then conditioned at 50% RH and 25 °C for 48 h prior to testing. The films were designated as starch/CNC, starch/CF, starch/CNC/CF, starch/VN, starch/CNC/VN, respectively, based on the types of GPE and presence of CNC (Table 1). The starch-glycerin film was prepared as a control.

2.3. Film characterization

2.3.1. Thickness and mechanical properties

Film thickness was measured to the nearest 2.54 μm with a hand-held micrometer (B.C. Ames Co., Waltham, MA, US). Five thickness measurements were taken for each film. Tensile strength (TS) and percent elongation at break (%E) were measured using a TA-XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY, US) based on the ASTM D882 method (1995). The initial grip separation was set at 50 mm and the crosshead speed was set at 200 mm/min. TS was calculated by dividing the maximum load (N) by the initial cross-sectional area (m^2) of the specimen.%E was calculated as the ratio of the final length at the point of sample rupture to the initial length of a specimen (50 mm) and expressed as a percentage. TS and%E tests were replicated five times for each film.

2.3.2. Water vapor permeability (WVP)

Water vapor transmission rate (WVTR) was determined gravimetrically using the method described by Gennadios, Weller, and Gooding (1994). Film specimens were mounted on polymethylmethacrylate cups containing 20 mL of distilled water. The cups were placed in an environmental chamber (Tenney Benchmaster Test Chamber) at 25 °C and 50% RH. A fan in the chamber was used to move the air at a velocity of approximately 200 m/min over the surface of the films to remove the permeating water vapor. The weights of the cups were recorded hourly for 8 h. Linear regression was used to estimate the moisture loss rate (g/h), and WVTR was calculated as loss rate/exposed area ($g{\cdot}h^{-1}{\cdot}m^{-2}$). Water vapor permeability (WVP) was calculated as

$$WVP = \frac{WVTR^{*}L}{\Delta p}$$

where L was the mean thickness of the film, and Δp was the difference in partial water vapor pressure between the two sides of the film (1.585 kPa).

2.3.3. Color and opacity

Film color was measured using a Minolta CR-400 chroma meter (Konica Minolta Camera Co., Ltd, Osaka, Japan) to determine 'L ' (lightness) from black (0) to white (100), 'a' [red (+a) to green (−a) color], and 'b' [yellow (+b) to blue (−b) color]. Color measurement was replicated five times in different points of the homogeneous film. Total color difference (ΔE) was calculated as

$$\Delta E = \sqrt{(L^{*}-L)^2+(a^{*}-a)^2+(b^{*}-b)^2}$$

where L*, a* and b* are the values for a white plate standard and L, a, and b are values from film samples.

Film opacity was determined spectrophotometrically based on Siripatrawan and Harte (2010). Film samples were cut into rectangle pieces and placed directly into a spectrophotometer (Evolution 60S,

Table 1
Composition of starch films.

Films	Grape type	Starch (% w/v water)	Glycerin (v/w% starch)	Grape pomace extract (v/v% film-forming solution)	Cellulose nanocrystal (v/v% film-forming solution)
Starch	–	5	30	–	–
Starch/CNC	–	5	30	–	10
Starch/CF	Cabernet Franc	5	30	8.0	–
Starch/CNC/CF	Cabernet Franc	5	30	8.0	10
Starch/VN	Viognier	5	30	8.0	–
Starch/CNC/VN	Viognier	5	30	8.0	10

– indicates not applicable.