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Effect of *Punica granatum* peel extracts on antimicrobial properties in Walnut shell cellulose reinforced Bio-thermoplastic starch films from cashew nut shells

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cashew nut shell
WNC
walnut shell cellulose
PP
pomegranate peel

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ABSTRACT

The main aim of the present study is to extract and characterize cashew nut shell (CNS) starch and walnut shell cellulose (WNC) for development of cellulose reinforced starch films. Moreover, the extraction and characterization of pomegranate peel extract, for incorporation with CNS-WNC films, was investigated. CNS starch was examined to be a moderate amylose starch with 26.32 ± 0.43% amylose content. Thermal degradation temperature of CNS starch was found to be 310 °C. Walnut shell cellulose was found to have high crystallinity index of 72%, with two thermal degradation temperatures of 319 °C and 461 °C. 2% WN cellulose reinforced CNS starch films were examined to have good oxygen transfer rate, mechanical and physical properties. Thermal degradation temperature of CNS-WNC starch films were found to be at the range of 298–302 °C. Surface roughness of CNS-WNC starch films were found to be increasing with increase in concentration of cellulose in films. Hydroxymethylfurfurole, Benzene, 2-methoxy-1,3,4-trimethyl and 1,2,3-Propanetriol, 1-acetate were found to be major active compounds present in hydrophilic extracts of *Punica granatum* peels. 2% WN cellulose reinforced starch films infused with hydrophilic active compounds of pomegranate peel was examined to be having good active package properties.

1. Introduction

All over the world, synthetic polymer-based composite materials are well established for a wide variety of applications (Sudharsan et al., 2016). With the increasing demand for fossil fuels, naphtha and natural gas accounts for about 4–5% of the world's oil consumption, for production of plastic resins. It is a challenge for the society to reduce the exploitation of fossil fuel, to protect the climate through the reduction of CO_2 release (Yuliana, Huynh, Hob, Truong, & Ju, 2012). Petrochemical plastics are of in high demand for several decades, thus their accumulation has become a major threat to society due to its non-biodegradable nature (Preetha, Sreekala, Matjaz, Miroslav, & Sabu, 2017). Over the past 20 years, more researches have been carried out to process the accumulated petro plastics, but complete solution for this problem has not yet been found. During last decade scientist and researchers have shifted their attention to find new renewable biodegradable sources to replace petro plastics, which can reduce their accumulation. Thus, in the future biodegradable plastics would play a big role as a viable alternative for petroleum-based plastics.

Biodegradable plastics can be produced from many renewable agricultural resources such as plant based polysaccharides, protein, lipids and polyesters. Polysaccharides serve as a major driving force in the field of bio plastic research. Due to their high abundance in nature, cellulose and starch, particularly, play vital role in bio plastic production (Sudharsan et al., 2016). Recent studies have used cellulose as a blending material with synthetic polymer to improve its synergistic thermal properties (Deepa et al., 2016; Iyer, Schueneman, & Torkelson, 2015; Mavelil-Sam et al., 2017; Maya, George, Jose, Sreekala, & Sabu, 2017; Yasir Beeran et al., 2016). Cashew (*Anacardium occidentale*) is an indigenous tree of Brazil and grows well in some countries in Asia and Africa. Cashew nut is considered as the major player in global market, due to its high demand in food sector (Maia, Andrade, & Zoghbi, 2000). Cashew nut comprise of edible kernel and inedible shell. Inedible shell of cashew is rich in unsaturated long chain phenols. However, after removing these phenols, defatted cashew nut shells contains high amount of starch (Yuliana et al., 2012). Thus, starch molecules present in cashew nut shells were expected to be a good film forming matrix for production of Bio-thermoplastic films. Walnut (*Julgans regia*) shell is the major waste material released from walnuts, after consuming its edible portion. Walnut shell powder was found to have good absorbing

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properties (Ding et al., 2013) and it is also found to be a good reinforcing material (Sarsari, Pourmousa, & Tajdini, 2016). Thus, cellulose molecules present in walnut shells were expected to be a good reinforcing material for cashewnut shell starch matrix to improve its thermoplastic capabilities. Since both cashew nut shell and walnut shells are considered as agricultural waste materials, they don't have any commercial value. Thus these agro waste materials, (rich in starch and cellulose) were selected for development of commercially valuable by-products (biodegradable packaging films).

Incorporation of packaging material with active compounds increases their antioxidant and antimicrobial properties (Chandra Mohan, Rakhavan, Sudharsan et al., 2016). This type of packaging materials serves as an active package for food products to improve its storage and retail shelf life (Chandra Mohan, Rakhavan, Radha Krishnan et al., 2016). Cashew nut shell starch films don't have high antimicrobial properties, due to inactivity of starch molecules against microbes. For production of active packaging films, active compounds should be incorporated in films separately. Recent works have also incorporated active ingredient rich waste materials such as turmeric and grape peels as active components in packaging films (Iyer, Zhang, & Torkelson, 2016). Pomegranate peels are inedible waste materials, rich in polyphenols and other active compounds and they are good source of antioxidant and antimicrobial compounds (Mohammad, Al-Jassani, & Hameed, 2016). Thus active compounds present in pomegranate peels are expected to improve the active packaging characteristics of cashew nut shell starch films, through their high antimicrobial and antioxidant properties.

The aim of the present study is to develop walnut cellulose reinforced cashew nut shell starch films. Moreover, isolation and characterization of walnut shell cellulose (WNC) and cashew nut shell starch (CNS) were investigated. It is also, the aim of this study to develop active packaging material by infusing pomegranate peel extract with WNC reinforced CNS starch films.

2. Materials and methods

2.1. Raw materials

Cashew nut shells were purchased from cashew nut production factory in Thoothukudi, Tamil Nadu, India. Walnut shell powder was purchased from Saloni enterprises, Ahmedabad, India. Pomegranate fruits were purchased from local market in Chennai, India, They were peeled to remove the peels for further process. All the analyses chemicals were purchased from local supplier for Merck Millipore (Merck specialities Pvt. Ltd.). Purity: EMPARTA^{ACS} grade for analysis. Microbial culture medias and media components were purchased from Hi-media laboratories.

2.2. Extraction methodologies

2.2.1. Starch extraction

Starch was isolated from samples using the method described by Yuliana et al. (2012). Cashew nut shell powder (10 g) was soaked in water with a ratio of 1:5 (w/w) for 3 h at 30 °C. The mixture was blended for 5 min and screened using a 60-mesh sieve. The residue was re-blended with 50 mL 70% ethanol for 5 min, passed through a 60-mesh sieve and then the residue was re-blended with 50 mL 0.1 M NaOH for another 5 min, and screened using a 60-mesh sieve. The filtrates obtained were combined and centrifuged at 11,000 rpm for 15 min. The supernatant was then decanted carefully and the residue was re-slurried with 100 mL of water, re-filtered twice through a 200 mesh screen and a Whatman analytical grade no.5 filter paper with 2.5 μm pore size and then washed successively with 0.1 M NaOH and deionized water. The residue restrained at the filter paper was dried by using a freeze drier. The dried starch was kept at −5 °C prior to analysis.

2.2.2. Cellulose extraction

Walnut shell (25 g) powder was treated with NaOH (750 mL, 0.5 M) for 2 h at 95 °C with continuous stirring and dark slurry was obtained by filtration. Then the slurry was washed several times with distilled water and dried. To remove γ and ß cellulose further digestion was done. Dried powder was mixed with 4 L of 17.5% aqueous solution of NaOH for 1 h at 80 °C and washed with distilled water. Remaining solids were filtered off and oven dried at 60 °C for 16 h. To bleach the obtained cellulose, it was soaked in stainless steel vessel containing 625 mL of 3.2% aqueous solution of sodium hypochlorite solution for 1.5 h at 40 °C, and washed with distilled water until obtaining neutral pH. Then it was filtered and oven dried at 60 °C for 16 h. Dried powder was milled in blender and sieved in 212 μm size sieve. Then it was again dried at 60 °C for 1 h and stored for further analysis (Wulandari, Rochliadi, & Arcana, 2016).

2.2.3. Active compounds extraction

Finely-powdered pomegranate peel (15 g) was separately blended for 2 min with 100 mL of solvents [Ethanol (E), methanol (M) and distilled water (W)]. These mixtures were then left in the shaker, at room temperatures for 16 h, then filtered using Whatman no. 1 filter paper and centrifuged at 6000 rpm for 10 min. The clear extracts were membrane filtered (0.45 μm) and then concentrated. Dried extracts of ethanol (E), methanol (M) and water (W) were dissolved in respective solvents and stored in −20 °C (10 mg/mL) (Chandra Mohan, Rakhavan, Sudharsan et al., 2016).

2.3. Film preparation

2.3.1. Starch based film

The film-forming solution was prepared by dissolving CNS starch (6%–10%) in 100 mL distilled water with gentle heating (about 40 °C) and magnetic stirring, followed by addition of 1.25% w/w glycerol as plasticizer. The dispersion was then heated at 90 °C for 20 min with gentle magnetic stirring to allow complete gelatinization of the starch. After gelatinization, the film solution was cooled to room temperature with gentle magnetic stirring for 30 min to reduce air bubbles. All the films were prepared by casting method, where 30 mL of filmogenic suspensions were poured onto Petri dishes (15 cm in diameter). Films were formed by drying at 40 °C in an incubator until reaching constant weight (about 24 h). The prepared films were peeled-off from Petri dishes and stored for further examination (Sudharsan et al., 2016).

2.3.2. Starch and gum based film

The film-forming solution was prepared by dissolving CNS starch (7.5%) and gums (Acacia Gum [7 & 8%], Guar Gum [0.25 & 0.5%] and Xanthan Gum [0.25 & 0.5%]) with varying concentration in 100 mL distilled water. Films were produced by following the method discussed in Section 2.3.1.

2.3.3. Starch citric acid cross-linked cellulose reinforced film

The film-forming solution was prepared by dissolving CNS starch (7.5%), Citric acid (1.2%) and varying concentration of cellulose (1%–5%) in 100 mL distilled water. The starch was gelatinized at 90 °C with gentle and magnetic stirring. Cellulose was dissolved in distilled water at 95 °C. Thereafter, citric acid was added to the cellulose solution at 80 °C. This solution was then added to gelatinized starch with stirring for 30 min, followed by addition of 1.25% w/w glycerol as plasticizer. Films were casted by following the method discussed in Section 2.3.1.

2.3.4. Pomegranate peel extract incorporated film

The film-forming solution was prepared by dissolving CNS starch (7.5%), Citric acid (1.2%) and cellulose (2%) in 100 mL distilled water with gentle heating (about 40 °C) and magnetic stirring, followed by addition of 1.25% w/w glycerol as plasticizer. The dispersion was then