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SHORT COMMUNICATION

Reinforced cassava starch based edible film incorporated with essential oil and sodium bentonite nanoclay as food packaging material

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Abstract Biodegradable packaging in food materials is a green technology based novel approach to replace the synthetic and conventional packaging systems. This study is aimed to formulate the biodegradable cassava starch based films incorporated with cinnamon essential oil and sodium bentonite clay nanoparticles. The films were characterized for their application as a packaging material for meatballs. The cassava starch films incorporated with sodium bentonite and cinnamon oil showed significant antibacterial potential against all test bacteria; *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus.* Antibacterial effect of films increased significantly when the concentration of cinnamon oil was increased. The cassava starch film incorporated with 0.75% (w/w) sodium bentonite, 2% (w/w) glycerol and 2.5% (w/w) cinnamon oil was selected based on physical, mechanical and antibacterial potential to evaluate shelf life of meatballs. The meatballs stored at ambient temperature in cassava starch film incorporated with cinnamon oil and nanoclay, significantly inhibited the microbial growth till 96 h below the FDA limits (10^6 CFU/g) in foods compared to control films that exceeded above the limit within 48 h. Hence cassava starch based film incorporated with essential oils and clay nanoparticles can be an alternate approach as a packaging material for food industries to prolong the shelf life of products.

Keywords Cassava starch films · Cinnamon essential oil · Green technology · Antimicrobial films

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Introduction

Conventional packaging materials are widely used in food industry due to cost effectiveness, strength, durability and ease of processing. The lack of degradability of polyethylene based conventional packaging materials leads to many environmental hazards (Debiagi et al. 2014). The conventional packaging system is possible to be replaced by biopolymeric films to reduce the environmental hazards. The concept of antimicrobial food packaging is gaining interest with aim to extend the shelf life of food products by preventing the microbial growth (Campos-Requena et al. 2017).

Cassava (*Manihot esculenta* crantz) is one of the most economical source to produce cassava starch that offers better film forming and casting properties due to high amylose content (17% w/w). The addition of plasticizers like glycerol, sorbitol and other polyhydroxy compounds to cassava starch films, improves the brittleness and mechanical properties of the film (Tongdeesoontorn et al. 2011). Moreover, starch based films are transparent, tasteless, odorless and do not interfere with the sensory parameters of the food product (Chiumarelli et al. 2010).

The health hazards associated with synthetic antimicrobials have increased the demand for natural antimicrobial components (Sadiq et al. 2015). Plant essential oils serve as a natural and safe source of antimicrobial compounds. Several investigations have been reported for the antimicrobial activity of edible films incorporated with essential oils against molds and bacteria (Souza et al. 2013). The addition of oregano essential oil improved the flexibility without affecting the thermal stability of the edible film (Pelissari et al. 2009). The fenugreek seed gum based nanocomposite films were reported to exhibit strong antibacterial properties against *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Bacillus cereus* (Memis et al. 2017).

The mechanical properties of starch-based films can be improved by cross-linking, blending with other polymers, preparing composite films and combination of blending with composite film preparation (Mbey et al. 2012). The composites of clay and starch presented the improved physical and mechanical properties of the starch-based films (Chiou et al. 2007). Clay is a natural toxin-free mineral that exhibits potential application in food, medical, cosmetic and healthcare (Chen and Evans 2005). Kashiri et al. (2017) reported that water vapor permeability, tensile strength, and Young's modulus values of active zein films were improved by incorporation of 2% (w/w) sodium bentonite clay. The objective of this study was to improve the shelf life of meatballs by packaging in cassava starch based films incorporating essential oils and nanoclay composites.

Materials and methods

Materials and reagent

Cassava starch was bought from Roi Et Flour Co., Ltd, Thailand and pork meat balls were purchased from local market of Pathumthani, Thailand. Analytical grade sodium bentonite nanoclay was supplied by Thai Nippo Chemical Industry Co., Ltd., Thailand. Cinnamon essential oil was purchased from Thai—China Flavours and Fragrances Industry Co., Ltd., Thailand. The microorganism (*Escherichia coli*, *Salmonella* and *Staphylococcus aureus)* used in this study were obtained from Thailand Institute of Scientific and Technological Research (TISTR). All other chemicals and reagents used in the study were of analytical grade.

Determination of minimum inhibitory concentration (MIC) of essential oils

The minimum inhibitory concentrations of cinnamon oil against *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* were determined using broth dilution method as described by Kubo et al. (2004). A series of two-fold dilution (16–0.25% v/v) of essential oil was prepared in DMSO. The cultures of target organisms were adjusted to 0.5 McFarland standard and diluted in sterile medium (1:100). The inoculum was then introduced in each concentration of essential oil. The test tubes were incubated at 37 °C for 24 h and the lowest concentration which had no visible growth was considered as the MIC.

Preparation of films

Different types of cassava starch based films were prepared by casting technique following the method of Kechichian et al. (2010) with slight modifications. Cassava starch based film incorporated with essential oil was made by mixing cassava starch (5%, w/v), glycerol (2.5%, w/w) and water (92.5%) and gradually heated on the hot plate with continuous stirring until the temperature reached to 55 °C. Different concentrations (1.5, 2 and 2.5%) of essential oil were added gradually with simultaneous stirring at 1200 rpm. The mixture solutions were then heated to 70 °C with continuous stirring and maintained for 20 min in water bath. The solution was casted over petri dish and placed into the vacuum drying oven at 50 °C for 30 min followed by drying at 50 °C for 24 h in the hot air oven and the films were then peeled off.

Cassava starch film incorporated with sodium bentonite was prepared as described by Kampeerapappun et al. (2007) with some modifications. Cassava starch and sodium bentonite nanoclay were physically mixed. Water and glycerine were then added and mixed by stirring at 1200 rpm. Subsequently, this solution was heated at 70 °C for 20 min with continuous stirring and casted over petri dish and finally dried. Different concentrations of glycerol (2 and 3%, w/w) and sodium bentonite (0.25, 0.5 and 0.75%, w/w) were used.

Cassava starch film incorporated with essential oil and sodium bentonite was prepared by mixing cassava starch and sodium bentonite followed by the addition of water and glycerol (2%, w/w). The resultant solution was heated to 55 °C with stirring by using magnetic stirrer. The essential oil was added gradually with continuous stirring using the mixer at 1200 rpm. The mixture was heated until the temperature was 70 °C and maintained for 20 min. Finally, the mixture was casted over petri dish and dried. The films made with only cassava starch were considered as control. All dried films were kept in the plastic bags and stored in the desiccators at 30–40% relative humidity (RH) for further study.

Evaluation of physical, mechanical and antibacterial properties of films

Film thickness

Hand micrometer (Miyuyoyo, Japan) was used to determine thickness of films by following the method as described by Rodríguez et al. (2006). The thickness of films was calculated from the mean of five random positions all over the films.

Water vapor permeability

Water vapor permeability (WVP) of film was measured based on ASTM method as described by Pranoto et al. (2005) with some modifications. A cup (5.5 cm in diameter and 3.4 cm in depth) containing 55 ml of distilled water was covered with a test film and then placed in desiccators filled with silica gel. The environmental temperature was maintained at 25 °C and the relative humidity (RH) inside desiccators was measured hygrometer. The WVP was calculated by following Eq. (1):

$$\text{WVP} = \frac{(\Delta w).x}{A.(\Delta t).(p_2 - p_1)} \quad \left(\text{g mm/m}^2 \text{ day kPa}\right) \tag{1}$$

where; Δw = the weight of water absorbed in the cup (g), Δt = time for weight change (day), A = area of the exposed film (m^2), x = film thickness (mm), p_2-p_1 = vapor pressure differential across the film (kPa), and calculated based on relative humidity and temperature inside and outside the cup.

Tensile strength and elongation at break analysis

The tensile strength (TS) and elongation at break (EB) of film were measured by using Lloyd Instrument Testing Machine type LRX 5 K. The method was followed as described by Pranoto (2004). Films were cut into 1.5 × 6 cm strips and held parallel with an initial grip separation of 3.5 cm. The film was pulled apart at 50 mm/min head speed. Then TS was calculated by using the Eq. (2):

$$\text{TS} = \frac{F_{max}}{x.d} \tag{2}$$

where F_{max} = maximum force at break, N, x = wide of film (m), d = thickness of film (m).

The following formula (Eq. 3) was used to calculate EB of film.

$$\text{EB} = \frac{H.t}{l} \times 100 \tag{3}$$

where; H = head speed, 50 mm/min, t = time for film extension until break (min), l = initial length of film (m).

Antimicrobial activities

Agar diffusion assay was used to determine the antibacterial activities of the films according to method of Sadiq et al. (2017) with some modifications. The films were cut into 6 mm discs (sterilized by UV light for 1 h) and placed on petri dishes containing Mueller–Hinton agar which were previously swabbed with prepared cultures of 0.5 McFarland standard. The plates were incubated at 37 °C for 24 h. The diameter of inhibition zones around the films was measured.

Effect of cassava starch based biodegradable film on shelf life of pork meatballs

The film with the best physical and antimicrobial potential was selected as biodegradable packaging for pork meatballs to extend the shelf life. Films were cut into square of 6 × 6 cm. Two sheets of film were sealed from 3 sides to make packaging for pork meatball (8 g). The packaging was closed by sealing the last side of packaging.

Two temperatures; ambient temperature (25 °C) and refrigeration temperature (4 °C) were used to store pork meatballs and quality of the pork meatballs was evaluated after every 2 days up to 10 days for 25 °C and after every 3 days till 21 days for 4 °C.

At each time interval pork meatballs were mixed with 0.1% peptone water and stomached in a stomacher (Stomacher 400, Lab. Blender, London, UK) for 2 min. A series of dilutions was prepared, and 0.1 ml of each dilution was spread directly on the plate containing plate count agar followed by incubation at 37 °C for 24–48 h (Georgantelis et al. 2007). Total colonies were counted and expressed in CFU/g pork meatball. Moreover, oxygen permeability rate of film was measured at 23 °C and 0% RH conditions by following the method of Hong and Krochta (2006) with some modifications. This method was based on D3985 of the American Society of Testing and Materials by using OX-TRAN 1/50 (Minneapolis, MN, USA). The test film was placed on a stainless-steel mask with an open testing area of 5 cm^2. The flow of nitrogen gas was contacted to one side of the film and the other side was contacted to the flow of oxygen gas. The relative humidity and temperature was maintained on the both sides of the film. The system was programmed to have a 10 h waiting period to allow the film to achieve equilibrium with controlled RH and temperature. Oxygen transmission rate was divided by oxygen partial pressure and multiplied by the thickness of film to obtain the oxygen permeability.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) and Tukey test to determine significant group differences ($p < 0.05$) between samples by using SPSS statistical software package (SPSS, version 23.0, USA).

Results and discussion

Minimum inhibitory concentrations (MICs) of cinnamon essential oils

The results showed that essential oils had antibacterial activities against tested pathogenic bacteria. MICs of cinnamon oil against *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus* were 0.5, 1 and 0.5% respectively. Oussalah et al. (2007) reported the antibacterial effect of cinnamon oil against *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus* with lower MIC values than this study.

Physical, mechanical and antibacterial properties of cassava starch based films

The physical properties, mechanical properties and antibacterial activities of the cassava starch based films were summarized in Tables 1 and 2. The essential oils were incorporated into cassava starch based films to improve the antibacterial activity.

Water vapor permeability (WVP)

WVP of films was decreased with increase in cinnamon oil concentration. The hydrophobic nature of essential oils affected the hydrophilic/hydrophobic property of cassava starch films and the water vapors transfer occurs through the hydrophilic portion of the films (Ojagh et al. 2010).

The concentration of glycerine and sodium bentonite significantly affected ($p < 0.05$) the WVP of the cassava starch films as shown in Table 1. The increment in the concentration of sodium bentonite from 0.25 to 0.75% (w/w) resulted in the significant decrease in water vapor permeability of composite films. This result corroborated with the previous report of Kampeerapappun et al. (2007), who reported a decrease in water vapor permeability of cassava starch composite films from 2000 to 1284 g m^{-2} day^{-1} with the increase of montmorillonite clay contents from 0 to 20 wt%. Based on the observations the best suited cassava starch based film was formulated by using glycerine (2%), different concentrations of essential oil (1.5, 2 and 2.5%) and sodium bentonite clay (0.75%).

WVP of composite film made up of cassava starch, sodium bentonite, glycerol and cinnamon oil was increased significantly ($p < 0.05$) with the increase in the concentration of cinnamon oil from 73.21 to 203.09 g mm/m^2 day kPa. The reported increase in WVP might be due to increase in hydrophilic content of the film or interactions of cassava starch, clay and essential oil might increase the free volume by reducing the intermolecular bonds between the polymers. The water or hydrophilic content acts as plasticizer in polymer matrix and increase the free volume, thereby facilitating the transfer of water vapor through the film (Cisnero-Zeballos and Krochta 2002).

Tensile strength

The mechanical properties explain the resistance of films to the normal stress that occurs in application, transportation, and handling to maintain the integrity and properties of foods (Pelissari et al. 2009). In this study, the addition of cinnamon oil and clay particles significantly affected the tensile strength of films. The tensile strength of films significantly ($p < 0.05$) decreased (0.31 MPa) with the addition of cinnamon oil compared to control film (1.68 MPa). Whereas, tensile strength of films increased with increasing concentration of clay particles. Similar results were reported for cassava starch-chitosan films incorporated with oregano essential oil (Pelissari et al. 2009). Memis et al. (2017) reported that the tensile strength of fenugreek seed gum based nanocomposite film was significantly increased after incorporation of nanoclay up to 5%.

Elongation at break

Incorporation of cinnamon oil in the cassava starch film significantly affected ($p < 0.05$) the elongation at break of the films by increasing the elongation at break of films (control film comprised of starch only) from 92.38 to 281.06%. Similar results were reported by Rojas-Graü et al. (2007) for alginate-apple puree edible film incorporated with oregano oil. Whereas, increasing concentration of essential oil resulted in decrease in elongation at break of cassava starch films incorporated with nanoclay composites.

Antibacterial activities

The cassava starch films with different concentration of sodium bentonite and glycerin did not show any antibacterial activity against *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*.

The cassava starch films incorporated with sodium bentonite and cinnamon oil demonstrated antibacterial

Table 1 Physical and mechanical properties of cassava starch films incorporated with cinnamon oil and nanoclay at different concentrations

Films	Concentration (% w/w)			Physical properties		Mechanical properties	
				Thickness (mm)	WVP (g mm/m^2 day kPa)	Tensile strength (MPa)	Elongation at break (%)
Control	–			0.29 ± 0.02^{c}	137.04 ± 19.56^{bcde}	1.68 ± 0.10^{ab}	92.38 ± 25.54^{bc}
	Cinnamon oil						
Films with essential oil	1.5			0.34 ± 0.01^{abc}	143.20 ± 18.58^{bcd}	0.34 ± 0.02^{c}	248.34 ± 26.93^{a}
	2			0.34 ± 0.02^{abc}	100.73 ± 12.33^{fg}	0.38 ± 0.04^{c}	270.82 ± 37.05^{a}
	2.5			0.38 ± 0.02^{a}	107.71 ± 13.73^{defg}	0.31 ± 0.05^{c}	281.06 ± 40.56^{a}
	Glycerine	Sodium bentonite					
Films with sodium bentonite	2	0.25		0.31 ± 0.01^{c}	133.06 ± 18.11^{bcdef}	1.43 ± 0.31^{b}	94.39 ± 18.68^{bc}
	2	0.5		0.32 ± 0.02^{bc}	97.68 ± 4.78^{fg}	1.68 ± 0.29^{ab}	73.62 ± 12.06^{c}
	2	0.75		0.34 ± 0.03^{abc}	73.21 ± 9.30^{g}	2.15 ± 0.65^{a}	67.72 ± 14.27^{c}
	3	0.25		0.33 ± 0.01^{abc}	126.52 ± 36.58^{cdef}	0.36 ± 0.05^{c}	147.29 ± 56.95^{b}
	3	0.5		0.33 ± 0.03^{abc}	117.63 ± 15.60^{cdef}	0.38 ± 0.06^{c}	120.59 ± 28.61^{bc}
	3	0.75		0.37 ± 0.01^{ab}	104.90 ± 16.64^{efg}	0.52 ± 0.16^{c}	99.41 ± 39.10^{bc}
	Glycerine	Sodium bentonite	Cinnamon oil				
Films with sodium bentonite and essential oils	2	0.75	1.5	0.32 ± 0.02^{bc}	146.46 ± 9.53^{bc}	0.63 ± 0.09^{c}	108.85 ± 7.29^{bc}
	2	0.75	2	0.34 ± 0.03^{abc}	165.01 ± 6.46^{b}	0.54 ± 0.02^{c}	96.62 ± 16.56^{bc}
	2	0.75	2.5	0.33 ± 0.01^{abc}	203.09 ± 5.70^{a}	0.51 ± 0.18^{c}	72.79 ± 16.79^{c}

Different superscript (a, b, c, d, e, f and g) letters within a column indicate the significant ($p < 0.05$) difference between mean observations

Table 2 Antibacterial activities of cassava starch based films

Sample	Concentration (% w/w)			Inhibition zone (mm)		
				Escherichia coli	*Salmonella typhimurium*	*Staphylococcus aureus*
Control	0			–	–	–
	Cinnamon oil					
Cassava starch films with essential oils	1.5			8.33 ± 0.29^{c}	9.00 ± 0.50^{b}	10.00 ± 1.00^{a}
	2			12.17 ± 0.29^{b}	10.67 ± 1.04^{ab}	11.17 ± 0.29^{a}
	2.5			13.00 ± 1.32^{ab}	12.00 ± 1.00^{a}	11.67 ± 1.15^{a}
	Glycerine	Sodium bentonite	Cinnamon oil			
Cassava starch films with sodium bentonite and essential oils	2	0.75	1.5	10.38 ± 1.11^{bc}	10.38 ± 0.48^{ab}	10.20 ± 1.15^{a}
	2	0.75	2	11.00 ± 0.82^{bc}	10.76 ± 0.63^{ab}	10.38 ± 0.48^{a}
	2	0.75	2.5	15.25 ± 1.85^{a}	12.50 ± 1.25^{a}	10.75 ± 0.50^{a}

– indicates no inhibition. Different superscript (a, b and c) letters within a column indicate the significant ($p < 0.05$) difference between mean observations

properties against all three test bacteria; *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*. Inhibition zone of films increased significantly ($p < 0.05$) when the concentration of cinnamon oil was increased. At 2.5% cinnamon oil, the maximum zone of inhibition was obtained against *Escherichia coli* (15.25 mm) and minimum was for *Staphylococcus aureus* (10.75 mm).

Selection of suitable cassava starch based biodegradable film

The cassava starch film incorporated with 0.75% sodium bentonite, 2% glycerine and 2.5% cinnamon oil was selected based on physical, mechanical and antibacterial potential to evaluate shelf life of pork meatballs. The oxygen permeability of the selected composite film was found to be 3.58 mm cm^3/m^2 day KPa, which was higher than the whey-protein-coated plastic films, previously reported by Hong and Krochta 2006. The lower oxygen permeability is preferred for food packaging materials but oxygen-barrier layers in food packaging materials are typically expensive and consist of synthetic barrier polymers.

Application of selected edible packaging to prolong the shelf life of pork meatballs

The selected composite film was applied as packaging to evaluate the shelf life of pork meatballs. The effect of different types of packaging materials on the bacterial growth in pork meatballs was shown in Fig. 1. According to Food and Drug Administration (FDA), the maximum acceptable level of total plate count in meat is 10^6 CFU/g. The pork meatballs contained in plastic bag and cassava starch film (control) exceed the maximum allowable level within 48 h whereas, meatballs packed in cassava starch film incorporated with cinnamon oil and sodium bentonite remained below the maximum allowable level until 96 h. Therefore, incorporation of cinnamon oil into packaging based on cassava starch showed antimicrobial efficacy by lowering microbial growth. Nanocomposite films based on thermoplastic starch/montmorillonite containing essential oil constituents significantly inhibit the microbial growth in strawberries inoculated with *Botryits cinereal* (Campos-Requena et al. 2017).

When pork meatballs contained in three different packaging were stored at refrigeration temperature (4 °C), they did not reach the maximum permissible level for 21 days as shown in Fig. 1B. The pattern of microbial growth remained constant which may be because of storage temperature.

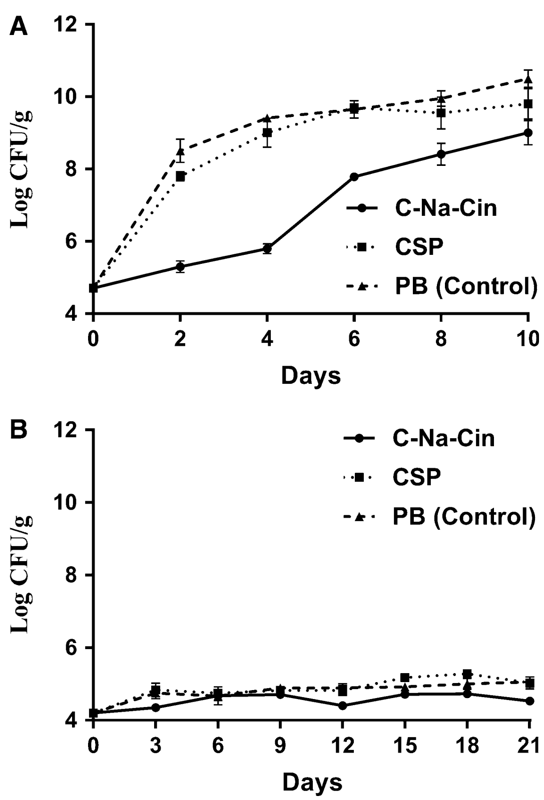

Fig. 1 Antimicrobial efficacy of different packaging films on microbial growth of pork meatball stored at ambient temperature (25 °C) (**a**) and refrigeration temperature (4 °C) (**b**). Where, C-Na-C = cassava starch film incorporated with essential oil and nanoclay, PB = plastic bag, CSP = cassava starch packing

Conclusion

Conventional packaging system can be replaced by biodegradable polymeric films to reduce the environmental hazards and prolong the shelf life of food products. The addition of essential oil and clay nanoparticles improved the antimicrobial effect of cassava starch film and significantly inhibit the microbial growth in pork meatballs compared to conventional packaging material. The cassava starch based films incorporated with essential oil and nanoclay are possibly to be used as packaging for food products due to their edible biodegradable and antimicrobial nature. In many of the developing countries, meatballs are used as street food and quality of food products can be maintained during transportation or in the absence of refrigeration conditions when stored in such edible and biopolymeric antimicrobial films.

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