Journal of Applied Pharmaceutical Science Vol. 7 (01), pp. 160-164, January, 2017
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2017.70122
ISSN 2231-3354

Development of Edible Biofilm Containing Cinnamon to Control Food-Borne Pathogen

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ARTICLE INFO

Article history:
Received on: 29/07/2016
Revised on: 19/09/2016
Accepted on: 23/11/2016
Available online: 31/01/2017

***Key words**:*
Cinnamon, Biodegradable edible films, Food packaging, Preservation, Antimicrobial activity.

ABSTRACT

Edible films of pectin and whey protein containing antimicrobials *i.e.* Cinnamic acid, Cinnamon leaf essential oil and Cinnamon bark powder were developed and functionally characterized. The cinnamon essential oil showed significant antimicrobial activity against the common food borne pathogens viz. *Escherichia coli*, *Staphylococcus aureus* and Listeria monocytogenes. The edible films were further structurally characterized for the transparency of visible and UV light, functional group characterization using FTIR analysis and determination of total moisture content. Antimicrobial activity of these films made from the edible source and cinnamon essential oil were further examined toward the associated food contaminants such as *E. coli, L. monocytogenes* and *S. aureus*. Edible films of pectin and whey showed significant level of antimicrobial potential against the food associated pathogens and could be used as a sustainable solution to the conventional packaging material specifically used in food processing.

INTRODUCTION

In current scenario, the biofilms or coatings have gained approval for the storage of foods, beverages, and medicines because of its ability to decrease pollution exerted by the commercial products made up of the polymers and are non-biodegradable (Malathi *et al.,* 2014). The additional benefits provide by the biofilms or coatings are related to the reduced cost and their ability to control moisture gases and lipid migration and also aids during the addition of additives and nutrients. Nowadays, the different polymers are used for synthesizing the edible films such polysaccharides, proteins and lipids or blend of either two of the polymers (Capitani *et al.*, 2016). Edible films synthesized are envisioned to elevate the mean life and lessen the chance of attack by the contaminants (McHugh *et al.,* 1994). Although many researches documented about distinct essential oils such as cinnamon and clove like fennel, cypress, lavender, thyme, herbs of cross pine and rosemary shows antimicrobial activity against certain types of bacteria which can be employed to prevent the food spoilage. The reports have been cited showing the antiviral, anti-toxigenic, anti-parasitic and insecticidal properties of these essential oils. The food industry has got highly interested with the idea of packaging material with antimicrobial activity, as the demand of consumer increases for the food product free from preservatives.

The use of polysaccharide such as pectin for the synthesis of biofilm provide**s** the properties such as water solvency and reduced water permeability (Xu *et al.*, 2005). On the other hand, whey protein shows properties like gas and mechanical barrier as compared to biofilms synthesized from polysaccharides. The molecular structure of whey protein displays high intermolecular binding potential (Jolie *et al.,* 2010). Biofilms, which are synthesized from these biopolymers are delicate and less manipulative by mechanical means. Thus, it has become essential to use the plasticizer to increase the flexibility and extensibility and additives are also added which facilitates as cross-linking agent. Whereas the excess amount of additives results in declination of flexibility, porosity, and permeability to gases, water vapours and solvents (Flores *et al.*, 2010).

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Nowadays, the awareness has been observed from consumer aspect, preferring healthy food provides health benefits. Thus, on this basis pectin and whey protein integrated with cinnamon products like cinnamic acid, cinnamon essential oil and cinnamon powder are utilized for synthesizing the edible biofilms. These edible biofilms on packaging interacts with the product as well as environment to increase adaptation phase whereas decline the proliferation rate of microbes. Currently, different plant essential oils are being extracted and integrated with biofilm components for evaluating their antimicrobial activity.

However, very less information is available about the plant essential oil as antimicrobial agents which can be used in combination for producing edible biofilms and coating. Main objective to conduct this study was to fabricate and characterized biofilm made from the edible source containing cinnamon as antimicrobial agent.

MATERIALS AND METHODS

Materials

Cinnamon powder (CP), Cinnamon essential oil (CEO) were attain from local market. Cinnamic acid (CA), pectin, glycerol and Tween 20 used in present study were of analytical grade. The bacterial strain of *Escherichia coli* and *Staphylococcus aureus* were used for determination of antibacterial activity were previously isolated from the market food samples.

Film Preparation

Whey protein of 8% w/v and Pectin 1% w/v concentration was used to formulate the edible films as follows; whey protein powder and pectin plus gelatin mixed and pH was adjusted to 8.0, after which it was heat at 90 oC for 30 min in a water bath. After that glycerol was added in 1:1 ratio. Later, Tween 20, at a level of 0.2% v/v, was added as an emulsifier to help dissolution in the film-forming solution. After 30 min of stirring, essential oils at 1%, 2%, 3% and 4% v/v concentration was added to the film-forming product. The solution was kept at room temperature for 30 min using a magnetic bead stirrer.

Five grams of the solutions was casted on 90 mm glass petri plates then dried for 72 h at 37 ^{o}C. Dried films were peeled from the plates and stored in a chamber at ambient temperature (Seydim and Sarikus, 2006).

Antibacterial Potential

To examine the antibacterial potential of with CP, CA, CEO and cinnamon powder, the pectin and whey protein film integrated CP, CA, CEO, by utilizing the agar diffusion technique. Inoculum of *E. Coli*, *L. monocytogenes* and *S. aureus* were inoculated separately and cultured it for overnight. Petri dishes with Muller Hilton agar were inoculated with the bacterial suspension with cotton swab. The five wells were made on the agar plate and 30µl solution by which film is to be formed was added into wells and incubated at 37 ˚C. Antibacterial potential was calculated by determining the inhibition zone

Characterization of the Films

Film Thickness

Thickness of whey and pectin films were estimated with the help of a digital micro meter of resolution 0.01mm on ten different positions. The mean value of the thickness was determined by the average of the thickness at different position Du *et al.* (2009).

Moisture Content

For estimating the moisture content, reduction in the mass of the films is observed when it is exposed to the 100ºC.The net variation between initial and final moisture content of the film provide statistics about the moisture content, according to the formula used by Soltani *et al.* (2014).

Moisture Content (%) = (I.M –F.M**)** *100/ I.M

Here, I. M = Initial Moisture and F. M = Final Moisture

Transparency determination

The transparency of the film was estimated with UV-Vis Spectrophotometer (Systronics Double Beam). The film was cut into long rectangular shapes (dimensions) and put in quartz cuvette, and absorbance was measured at the wavelength of 550nm. According to Han *et al*. (1997) transparency (T) can be evaluated with formula:

$T = A_{550}$/ film thickness

Here, A = absorbance of the film at a wavelength of 550 nm.

Fourier Transform Infrared Spectroscopy

For evaluating the interaction among the functional groups of pectin as well as whey protein with cinnamon oil was done with the use of Fourier Transform Infrared (FTIR) Spectroscopy at room temperature in scanning range of 500-4000 cm^{-1} **(**Pranoto *et al.*, 2005).

RESULTS AND DISCUSSION

Antibacterial Potential of different cinnamon variations

The antibacterial Potential was analysed for CP, CA and CEO. The maximum zone of inhibition was observed for the CEO of 5 % (v/v) concentration as compared to the CP and CA. The CEO showed effective inhibition zone against the *E. coli*, *S. aureus* and *L. monocytogenes* at 5 % (v/v) essential oil. Thus this minute volume of CEO exhibited the effective result were used for further analysis. For further assessment the CEO was integrated with the pectin and whey film. CEO was reported for significant antibacterial potential against pathogens. Bahram *et al*. (2014) reported CEO for their antimicrobial activities against various food contaminants like *B. subtilis*, *L. monocytogenes*, *E. coli* and *Candida albicans* at 1.5 % (v/v) concentration. Whey protein integrated with CEO which act as antibacterial compound while preparing the edible film. Films containing CEO displayed prominent antibacterial potential against food pathogens, and showed significant inhibitory outcome with *Candida albicans*. Antimicrobial activities of Cinnamon Oil and

Cinnamaldehyde showed significant inhibition of *S. aureus*, *E. coli*, *E. aerogenes*, *P. vulgaris*, *P. aeruginosa*, *V. cholerae*, *V. parahaemolyticus and S. typhymurium*, *C. albicans* and *Aspergillus* spp. (Ooi *et al.*, 2006). In present study, antimicrobial activities of CA and CP displayed poor antibacterial potential as correlated with CEO.

Table-1: Antimicrobial activity of the Cinnamon powder (CP), Cinnamic acid (CA) and Cinnamon essential oil (CEO) against food borne pathogens.

Film Forming Solution	Concentration of cinnamon oil (v/v)	Zone of Inhibition (mm) *E. coli*	*S. aureus*	*L. monocytogenes*
Cinnamic powder (CP)	**1%**	6.85±0.2	6.27±0.23	5.53±0.11
	2%	7.13±0.12	7.25±0.11	6.21±0.12
	3%	7.95±0.22	8.00±0.19	7.23±0.12
	4%	8.39±0.15	8.45±0.16	7.45±0.19
Cinnamic acid (CA)	**1%**	9.85±0.17	8.03±0.15	3.56±0.15
	2%	10.31±0.23	8.70±0.12	4.27±0.14
	3%	10.52±0.11	9.08±0.10	4.23±0.16
	4%	10.68±0.14	10.14±0.29	6.48±0.19
Cinnamon essential oil (CEO	**1%**	13.07±0.15	11.505±0.22	10.73±0.23
	2%	16.31±0.19	16.195±0.21	13.54±0.21
	3%	17.52±0.22	16.330±0.10	17.81±0.24

Antibacterial Potential of Pectin and whey films integrated with CEO

Antibacterial potential of edible films disks are presented in Table 2. No antibacterial activity was visualized on the control film disks which doesn't contains CEO against tested pathogens, which resemble with outcome attained by Bahram *et al*. (2014) with edible films formed from whey protein. Edible films of whey as well as pectin integrated with CEO showed significant inhibition against tested pathogens. Accumulation of CEO into whey and pectin resulted in dispersion of antibacterial essential oil by employing Mueller Hinton agar medium plates and delivered an inhibition zone adjacent the film disks for all the pathogens tested.

Various reports informed that CEO could suppress the proliferation of pathogenic microorganisms (Ouattara *et al*. 2000; Matan *et al.*, 2012; Valero and Salmeron 2003, Ojagh *et al*. 2010). The minimum zone of inhibition was observed for *E. coli* in edible film disks (15.55 and 11.66 mm for pectin and whey film disks respectively).

The maximal activity was observed for *S. aureus* and *L. monocytogenes* (26.39, 26.88 and 13.78, 14.56 mm respectively for edible films of pectin and whey comprising 5% (v/v) essential oil. Inhibition zone developed by edible disks were maximal in comparison to the edible disk, probably because of more dispersion of essential oils through the film wells in relation to pectin and whey edible film disks. Reports have been documented that antimicrobial activities were more effective against the gram positive bacteria as compared to the Gram negative bacteria (Sanchez-González *et al*., 2011).

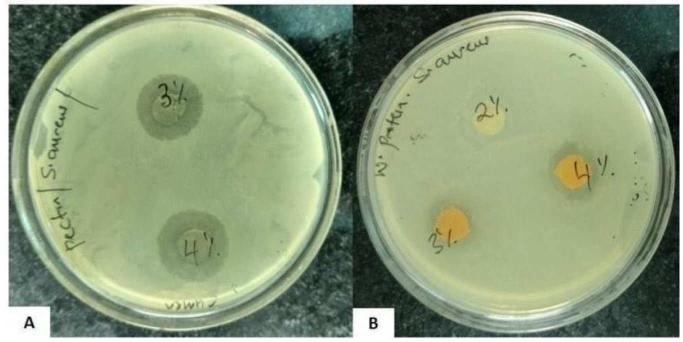

Fig. 1: Antimicrobial activity of the edible packaging films

Table-2: Antibacterial potential of the edible film of pectin and whey containing Cinnamon essential oil against food borne pathogens.

Edible Film with Cinnamon essential oil	Cinnamon oil (v/v)	Zone of Inhibition (mm) *E. coli*	*S. aureus*	*L. monocytogenes*
Pectin film	5%	15.55±0.12	26.39±0.23	26.88±0.10
Whey film	5%	11.60±0.10	13.78±0.21	14.56±0.13

Characterization of the Films

Film thickness

The biofilms synthesized from the pectin, whey protein blended with CEO were measured at 10 different positions with the digital micro meter (0.001 mm) to estimate the thickness of the biofilms (figure-1). Thus for estimating the physical as well as mechanical traits the arithmetic mean is employed. The thickness based on the weigh (in gm.) is represented in the table 3. The thickness of the pectin and whey edible films were increased with increased in amount of film forming solutions ranges from 0.124-0.249 and 0.136-0.200 respectively. The thickness of pectin and whey edible films did not exhibit any alteration on integration of CEO, but were in range of 0.125 to 0.127 and 0.127 to 0.247 mm respectively as represented in Table 3. Strengthening edible films of pectin and whey could be associated with the change in the microstructure during the interaction among the film and essential oil and which also grant something to mean thickness of the film. Bahram *et al*. (2014) also reported the similar results while incorporated CEO in whey protein edible films and observed that there was no significant transition in the thickness due to the integration of CEO ranging from 0.31 to 0.34 mm. The incorporation of essential oil did not change the thickness of the edible film considerably also the amount of essential oil incorporated attributed to the tensile strength of the edible film. Similar results were encountered in the antibacterial potential and physical traits of edible films integrated with polyphenols from the apple skin (Du *et al.*, 2011). The integration of polyphenols of apple skin contributes for strengthening of the edible films and associated with the cooperation among apple film and skin polyphenols. The thickness of apple edible films is directly proportional to polyphenols concentration.

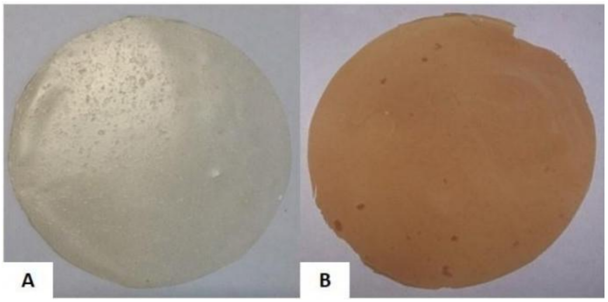

Fig. 2: Representation of edible films incorporating cinnamon essential oil (A) Pectin edible film (B) Whey protein edible film.

Table 3: Representation of thickness (in mm) with respect to weight of the biofilm

Films	Weigh (gm)	Thickness (mm)
Pectin	2.5	0.124 ± 0.03
	3.0	0.149 ± 0.02
	4.0	0.249 ± 0.05
Whey Protein	3.0	0.136 ± 0.04
	4.0	0.200 ± 0.02
Pectin + Cinnamon essential oil	2.5	0.125 ± 0.02
	3.0	0.151 ± 0.05
	4.0	0.253 ± 0.02
Whey + Cinnamon essential oil	2.5	0.127 ± 0.05
	3.0	0.154 ± 0.03
	4.0	0.257 ± 0.06

Moisture Content

Moisture content was calculated to determine the water affinity of the biofilm. The result displays that the PCO (4.69%) and WCO (5.75 %) has less moisture content as compared to control pectin (6.34%) and whey protein edible films (8.45%). The moisture content values of PCO and WCO edible films considerably reduced as it is integrated with CEO. That is further associated with hydrophobic traits of cinnamon oil. Packaging edible films have to sustain moisture content within the wrapped food product. For that reason, the assessment of edible films moisture content is important until active food packaging is concerned. Comparative analysis of the data was done on the emulsified fatty acid edible films as well as the protein pistachio

Decline in moisture content in protein-olive oil possibly be associated with protein-water exchange mechanism commenced in the emulsified films (Zahedi *et al.*, 2010; Rocca-Smith *et al.*, 2016).

Table 4: Moisture content determination of pectin and whey edible films.

Edible Film	Moisture Content (%)
Pectin film without cinnamon essential oil (control)	6.34
Whey protein film without cinnamon essential oil (control)	8.45
Pectin + Cinnamon Essential oil (PCO)	4.69
Whey Protein + Cinnamon essential oil (WCO)	5.75

Transparency determination

The opaque biofilms were analysed with UV-Vis spectrophotometer at two different wavelengths 280 nm and 550 nm for transmittance and UV opacity. The incorporation of CEO revealed decline in edible film opaqueness on comparison with control film (pectin and whey), implying regarding the transparency of the packaging films as it was integrated with CEO (Table-5). In spite of this, PCO and WCO edible films, the gelatin films showed sharp decrease in UV opacity even when it is incorporated with 5% CEO from 47.14, 48.32 to 6.46 and 6.32 respectively. Edible films transparency is the major concern while synthesizing food packaging material as it is directly correlated with the exterior of the packaged material. Transparency acts as an additional factor to exploit the constituents of edible film (Liu and Zhang, 2006). If empathy amongst edible film ingredients is not clear, thus induces hindrance in transmittance which is reduce because light gets reflected towards the two-phase interface (Rhim *et al.*, 2007).

Table-5: Film opacity (A550/mm) values for the control films and PCO and WCO edible films.

Edible films	Transmittance % (550nm)	UV (280nm)
Pectin	85.56	47.14
Whey protein	84.37	48.32
Cinnamon essential oil + Pectin	78.03	6.46
Cinnamon essential oil + Whey protein	76.76	6.32

Fourier Transform Infrared Spectroscopy

Spectra obtained from PCO and WCO edible films showed identical arrangement of peaks of absorption in the range of 3400 and 840 cm^{-1}. Spectral peaks within the range 3400 and 1020 cm^{-1} coincide with the -OH arrangement of functional group of hydroxyl, (C= O) bonded to aromatic group. Similar results were also reported when working on cinnamon incorporated edible films (Siripatrawan and Harte, 2010). Peak of the control as well as of the film integrated with 5 % CEO illustrated the notable spectral lines at 3,232/cm. The absorption bands perceived within 1,600 and 1,700/cm (1,627/cm) signify the existence of amid I bands (extension of C = O) (Andreuccetti *et al*. 2009).

CONCLUSIONS

Current study was focused on the scrutinizing the pectin as well as whey protein which are incorporated with the cinnamon oil as the antibacterial agents. The antibacterial potential of cinnamon oil was notably higher than cinnamon powder as well as cinnamic acid against *E. coli* and *S. aureus*. It was observed that integration of CEO with edible film doesn't hinder the permeability of water vapour and tensile property. FTIR provide the information related to the interaction between the functional groups of antimicrobial agent with biofilm polymers. The results signify the cinnamon essential oil as the efficient antimicrobial agent against pathogenic bacteria which can be used in the packaging material. Thus, it is essential to persist with this research to obtain edible biofilm with improved physical and biological properties. That can be employed in the packaging industry for coating the food such as the bakery product, dairy product, meat and vegetable without affecting their quality. The edible films development can be scaled up using low cost agriculture residues for as a casting polymers, which in turns can regulate the cost of production and would be an alternative to the conventional packaging material.

Financial support and sponsorship: Nil.

Conflict of Interests: There are no conflicts of interest.

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How to cite this article:

Sharma D, Dhanjal DS, Mittal B. Development of Edible Biofilm Containing Cinnamon to Control Food-Borne Pathogen. J App Pharm Sci, 2017; 7 (01): 160-164.