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PREPARATION OF DIFFERENT ZEIN FILMS FROM CORN GLUTEN MEAL AND THEIR USE IN COATING OF DIFFERENT MODEL FOODS

Sampy Duggal[1], Somesh Sharma[1], Rachna Verma[2] and Dinesh Kumar[1]*

[1*]School of Bioengineering and Food Technology, Shoolini University, Solan-173212, (H.P.) India.
[2]School of Biological and Environmental Sciences, Shoolini University, Solan-173212 (H.P.) India.

Abstract

In the present study, the zein (10% w/w, dry basis) isolated from corn gluten meal from *Bajaura Makka* (*Zea mays* L.) variety of H.P. was solubilised in hot ethanol (90%) using glycerol and fructose as plasticizers. The three types of zein films were prepared, first film preparation was done by mixing zein with absolute ethanol (90%), second film prepared was zein and ethanol added with glycerol 5% (w/w) as plasticizer and third was zein and ethanol added with fructose 4% (w/w). The various physiochemical properties of these three zein films *viz*. colour, thickness, solubility, water vapour transmission rate were evaluated. The thickness for zein ethanol film was 0.14±0.02 mm and the thickness of films prepared by zein ethanol glycerol mixture and zein ethanol fructose mixture were 0.22±0.04 mm and 0.19±0.07 mm respectively. The water vapour transmission rate of different zein films *i.e.* zein ethanol mixture, zein ethanol fructose and zein ethanol glycerol was observed as $0.54 \times 10^{-3,}$ 0.36×10^{-3} and 0.25×10^{-3} ($g/m^2/sec$) respectively and solubility of these zein films was 17±0.67%, 25±0.42% and 20±0.80% respectively. The color of zein ethanol film was 1.3 red units, 3.6 yellow units, 4.20 white units and color of zein ethanol fructose film was 1.2 red units, 10 yellow units, 7.4 white units and color of zein ethanol glycerol film was 1 red units, 4.7 yellow units, 4.1 white units as observed in Lovibond Tintometer. The results showed that the film prepared with combination of zein + ethanol + glycerol was having higher film thickness, water vapour transmission rate (WVTR) than other two zein films. These zein films were further used for coating of different model food products *viz*. Himalayan fig (*Ficus palmata*) leather, roasted nuts, chocolate cookies and vanilla cookies.

Key words: Zein, ethanol, fructose, glycerol, physiochemical characterization, model food coating.

Introduction

Zein (a valuable protein of *Zea mays* L.) is a major protein in corn meal and because of its ability to form coatings and films; it is attractive to the food and pharmaceutical industries (Lin *et al*., 2011; Wang and Padua, 2002). Zein films are generally prepared by dissolving zein in warm aqueous ethanol or isopropanol and pouring onto non stick substrates. Zein is soluble in different mixtures of alcohol and water and is casted on appropriate substrates (Gennadios *et al*., 1994; Lai and Padua, 1997). Films are formed through the development of hydrophobic, hydrogen and limited disulfide bonds between zein chains (Gennadios *et al*., 1994). Self-induced self-assembly mechanisms explained interaction of zein molecules to form films (Wang and Padua, 2010; Wang *et al*., 2008). Zein films are normally clear and brittle, thus requiring plasticization. For improving flexibility of zein films, plasticizers are added which acts as a lubricant between the molecular chains. Zein films that are plasticized with hydrophilic plasticizers *viz*. sorbitol, glycerol and fructose normally show increase in permeability to gases and water vapour (Ghanbarzadeh *et al*., 2006; 2007). Park and Chinnan, (1995) described detailed physiochemical properties of zein films and their evaluation. Zein films exhibits various properties and shows numerous of applications as transparent coating, glossy, grease proof, tasteless nature and resistance to microbial attack (Shukla and Cheryan, 2001). In the present study, zein films were prepared with mixture of ethanol and plasticizers, characterized and used for coating of different model foods.

***Author for correspondence :** E-mail: chatantadk@yahoo.com

Materials and methods

Preparation of different zein films

Zein was obtained through a green process using immobilized enzymes (protease and amylase) in a two stage glass bioreactor, from corn gluten meal (Duggal and Kumar, 2016). Further, recovered zein was used to optimize the preparation of zein films. The different types of zein films were prepared using Gannadios *et al*., *(*1993) method. First set of Zein film preparation was done by mixing zein (10% w/v) with absolute ethanol (90%). The second zein film was prepared by adding zein with ethanol (90%) and fructose 4% (w/w) as a plasticizer and third set of zein film was prepared using zein and ethanol with glycerol 5% (w/w) as a plasticizer. In all film preparations, the film forming mixture was heated from 60-70°C and maintained at 70°C for 10 min with regular stirring. Before casting zein solutions, these were sonicated with ultrasonic cleaner for 5 minutes. The different zein films were prepared by pouring 5 ml of each mixture, suspensions on polyethylene petriplates. The plates with the film solution were then dried at 60°C for 4 h in oven under air circulation. The dry films were peeled off from the trays and kept in airtight polyethylene bags.

Physiochemical characterization of different zein films

All three different types of zein films prepared with or without plasticizer were studied for their physiochemical properties (thickness, colour, solubility and water vapour transmission rate) using Gontard *et al.*, (1994) and American Society for Testing Materials (ASTM, 1995) methods.

Thickness of zein films

A digital vernier calliper was used to check the thickness of zein films, with two upper and two lower jaws. The lower jaws of the digital calliper was placed on the outside part of zein film and slide against the zein film until both of the lower jaws tighten against the outside surfaces of zein film perfectly. The film thickness was determined from the average measurements performed for each zein film.

Color of zein films

The color of different zein films was measured using Lovibond tintometer. Each film was taken in the clean cuvette to get accurate results. The tintometer contained complete set of 11 racks containing permanently coloured glass filter for Loviband colour scale (Red 0.1-0.9, 1.0-9.0, 10.0-70; Yellow 0.1-0.9, 1.0-9.0, 10.0-70; Blue 0.1-0.9, 1.0-9.0, 10.0-40; Neutral 0.1-0.9, 1.0-3.0). All different zein films were compared for colour results according to matching with lovibond colour scale.

Solubility of zein films

Solubility in water was defined as the percentage of the dry matter of film which is solubilised after 24 h., immersion in water. The solubility tests of zein films were performed using desiccators containing dry calcium sulphate. About 500 mg of each film was immersed in beakers containing 50 ml of distilled water at 23°C for 24h., with periodical gentle manual agitation. The zein films were removed from the water and were placed back in the desiccators until they reached a constant weight to obtain the final dry weight of the film. The percentage of total soluble matter (TSM%) of the film was calculated using following formula:

$$\text{Total soluble matter (TSM\%)} = \frac{\text{Initial dry weight- final dry weight}}{\text{Initial dry weight}} \times 100$$

Water vapour transmission rate (WVTR) of prepared zein films

Water vapour transmission rate of prepared zein films were measured using ASTM, (1995) modified method. Zein film was sealed to test cell containing 30 ml of distilled water. The test cell was then kept in a large plastic cup containing dehydrated silica gel. Silica gel should be dried at 180°C for 3 hour before the measurements. The whole assembly was kept at 25°C and weight loss of the test cell was measured after storage for 24 h. WVTR of the films was calculated using the following formula:

$$\text{WVTR (g/m}^2\text{/sec)} = \frac{\Delta W}{T \times A}$$

Where,

Δ W is the weight loss of the test cell

T is the time of storage

A is the area of exposed film

Measurement of the burst strength of different zein films

The Stable Micro System Texture Analyzer (Text Plus) was used to analyse the burst strength of different

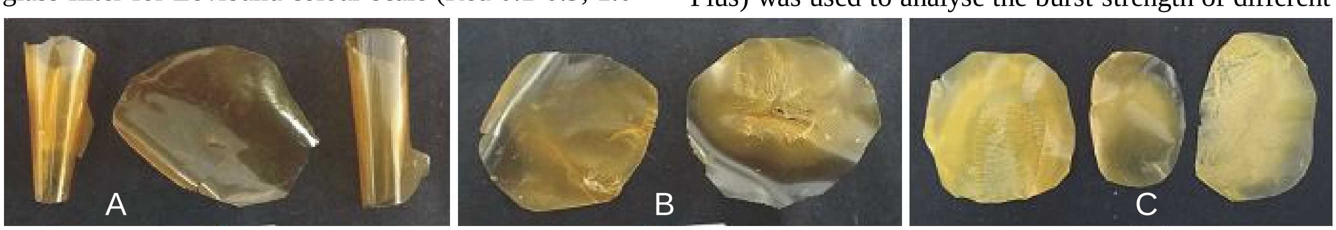

Fig. 1: Zein films preparation with zein isolates and different combination of ethanol and plasticizers. Zein films prepared with A. Zein (10%) + ethanol (90%); B. Zein (10%) + ethanol (90%) + fructose (4%) as a plastcizer and C. Zein (10%) + ethanol (90%) + glycerol (5%) as a plasticizer.

Table 1: Physiochemical characteristics of different combination of zein films prepared from corn gluten meal using *Bajaura Makka* variety of H.P.

Films Type & Combination	Film Thickness (mm)	Film Solubility (%)	WVTR ($g/m^2/sec$)	Colour in Lovibond Tintometer
Zein+Ethanol	0.14±0.02	17±0.67	0.54×10^{-3}	1.3 red units; 3.6 yellow units; 4.20 white units
Zein+Ethanol+Fructose	0.19±0.07	25±0.42	0.36×10^{-3}	1.2 red units; 10 yellow units; 7.4 white units
Zein+Ethanol+Glycerol	0.22±0.04	20±0.80	0.25×10^{-3}	1 red units; 4.7 yellow units; 4.1 white units
Values are expressed as means of triplicates (n=3 ± SD)				

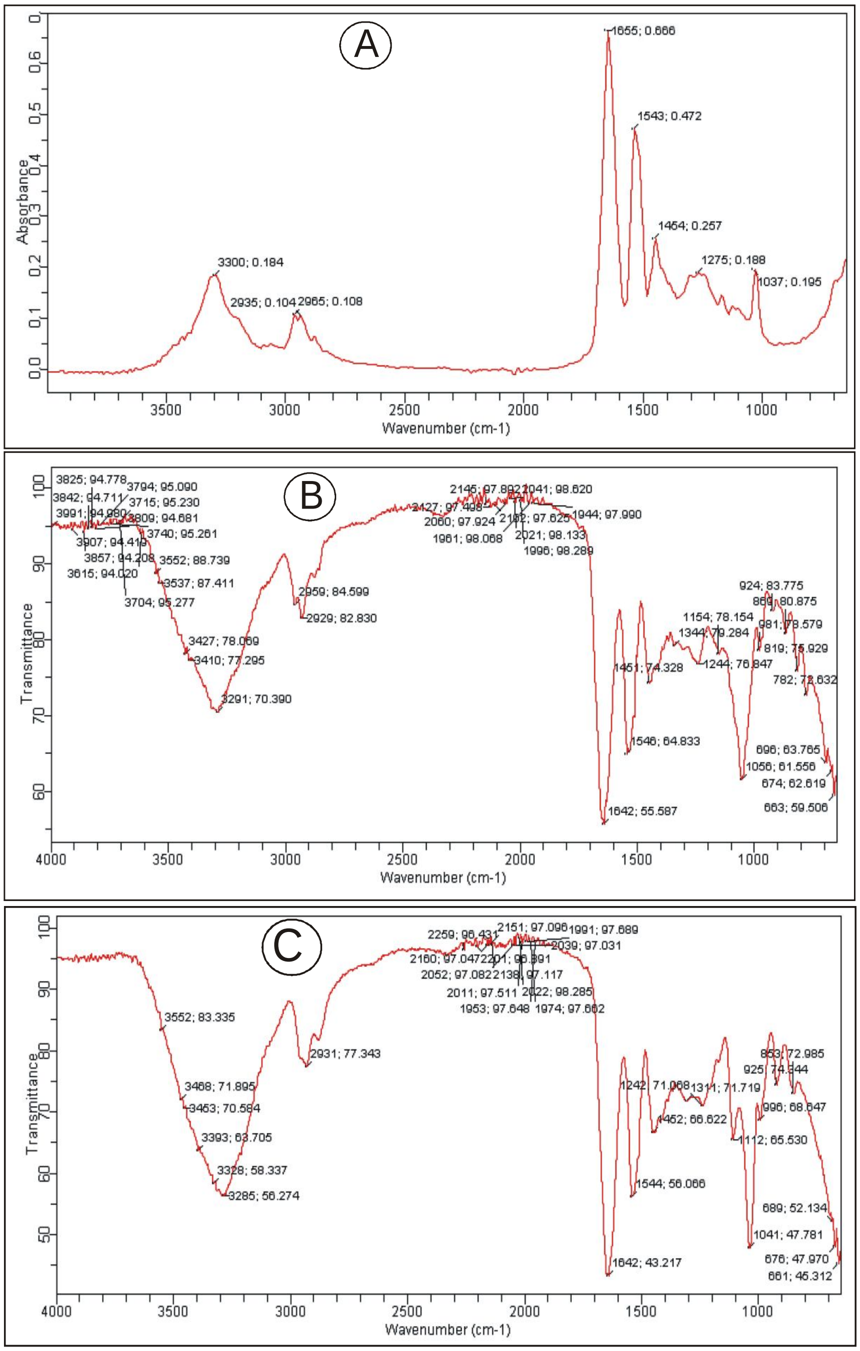

Fig. 2: FTIR spectra of different zein films prepared using. A. zein +ethanol; B. zein +ethanol + fructose and C. zein +ethanol + glycerol combinations. FTIR spectra showing aromatic bending of C=C at absorbance (cm^{-1}) 1555, carboxylic acid O-H stretch at 2965 cm^{-1} and amine N-H stretch of different zein films at 3300 cm^{-1}.

zein films (ASTM, 1989). Sheets of different zein films were cut into small squares of 30 mm × 30 mm each. Three samples of zein films each were tested to generate the results for mean burst strength (N) and mean distance to burst (mm) using texture analyzer.

FTIR analysis of different zein films

All the samples of zein films (Zein+Ethanol, Zein+Ethanol+Fructose and Zein+Ethanol+Glycerol) were stored in a desecrator containing silica gel for at least 48 h., at room temperature to ensure minimal moisture content before spectroscopic analysis using Agilent Technologies Cary 630 FTIR system. Film samples (10 mm × 10 mm) were placed and gently squeezed by FTIR screw to promote contact with the crystal. The spectra were collected with frequency range of 4000-500cm^{-1} (Soliman *et al*., 2012).

Coating of various model foods using prepared zein films

After the characterization of zein films, all films were used for the coating of various model foods *viz*., Himalayan fig (*Ficus palmata*) leather, roasted almond nuts, chocolate cookies and vanilla cookies. The Himalayan fig leather pieces were coated by dipping them in three different sets of solution *i.e*. a) zein (10%) + ethanol (90%), b) zein (10%) + ethanol (90%) + fructose (4%) as plasticizer and c) zein (10%) + ethanol (90%) + glycerol (5%) as plasticizer respectively. The coating of other model foods *viz*. roasted almond nuts, chocolate cookies and vanilla cookies, was done with similar combinations of zein film solutions as discussed above for Himalayan fig leather.

Table 2: Stability test results obtained with different zein films combinations prepared using Texture Analyzer.

Film Sample Type	Mean Burst strength (N)	Mean Distance to Burst (mm)
Zein+Ethanol	19.91±9.69	1.64±0.28
Zein+Ethanol+Fructose	17.02±5.47	3.42±0.51
Zein+Ethanol+Glycerol	7.46±2.33	2.20±0.40
Values are expressed as means of triplicates (n=3 ± SD)		

Results

Preparation of different zein films

The different types of films were prepared with zein obtained from corn gluten meal (CGM) from *Bajara Makka* of H.P. The results of zein films prepared are shown in fig. 1. The films prepared by zein + ethanol combination were brittle, clear and non-flexible and non-stretchable in shape and structure as shown in fig. 1A. These zein films were not stable and were easily breakable with folding. The film prepared by zein + ethanol + fructose combination (Fig. 1B) were thick, less clear, less brittle and more flexible and stretchable then the first combination. The third type of zein film was prepared by dispersing zein in ethanol + glycerol as a plasticizer. These zein films (Fig. 1C) were more thick, flexible, stretchable, less clear and less brittle than the first two combinations. The uses of glycerol as plasticizers act as a lubricant between molecular chain of zein and increase the flexibility of the zein film.

Physiochemical characterization of different zein films

Various physiochemical properties (thickness, colour, solubility and water vapour transmission rate) were evaluated for pure zein films and zein films with combinations with fructose and glycerol as plasticizers. The results of this physiochemical characterization are shown in table 1. In characterization of different zein films, the highest thickness (0.22±0.04 mm) was observed in zein + ethanol + glycerol film and lowest thickness (0.14±0.02 mm) was observed in zein + ethanol film. The highest solubility (25±0.42%) was observed in zein + ethanol + fructose films and lowest solubility (17±0.67%) was observed in zein + ethanol films. The highest water vapour transmission rate was shown in zein + ethanol film *i.e.* 0.54×10^{-3} (g/m^2/sec) and lowest was recorded in zein + ethanol + glycerol film *i.e.* 0.25×10^{-3} (g/m^2/sec). The Lovibond tintometer was used for estimation of colour of different zein films and the results of this study are also shown in table 1. The highest units of colours was seen in zein + ethanol + fructose film (1.2 red, 10 yellow and 7.4 white units) and lowest was

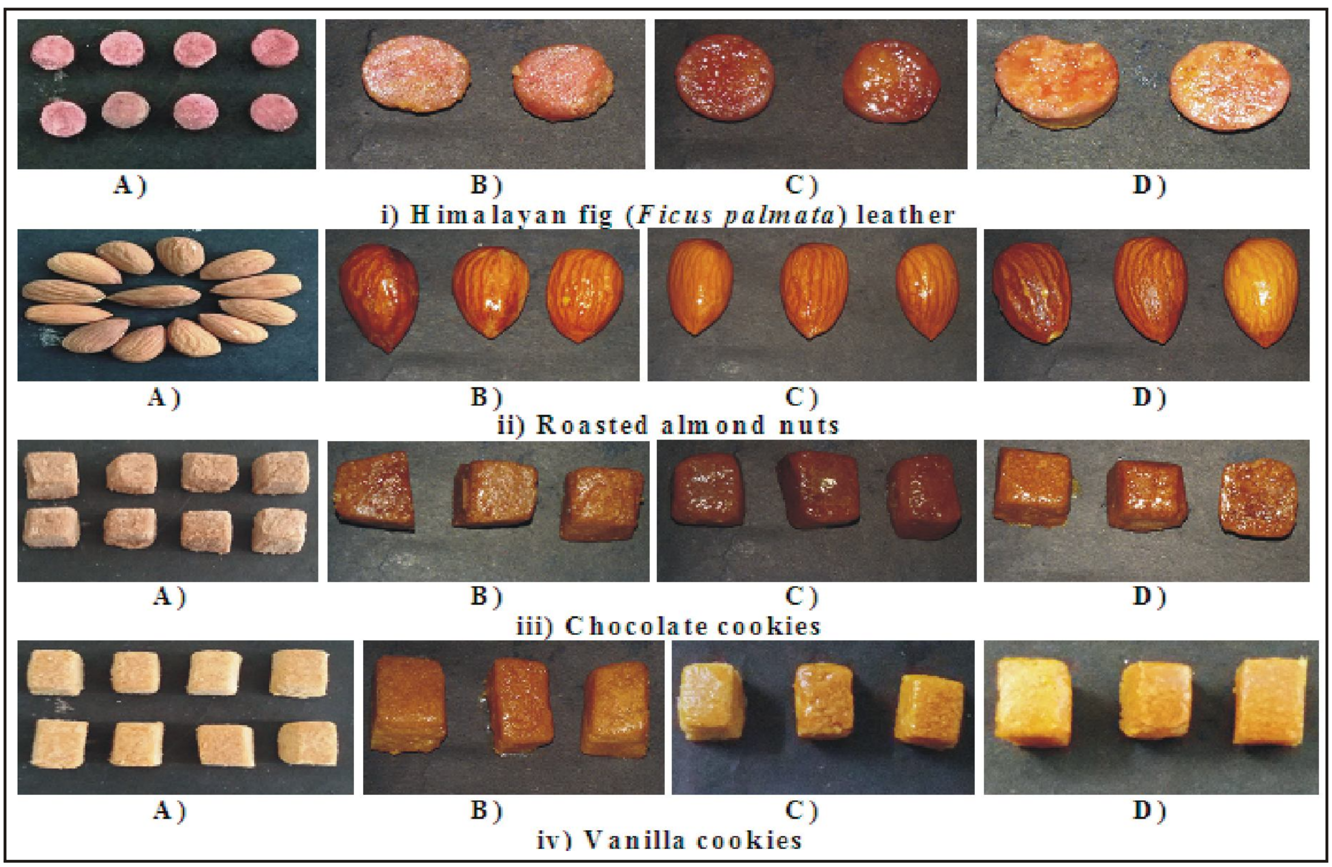

Fig. 3: Coating of model food products with different zein films. A. Model food products without coating as control; B. Coating with Zein + ethanol; C. Zein + ethanol + fructose as plasticizer and D. Zein + Ethanol + glycerol as plasticizer.

observed in zein + ethanol + glycerol film (1 red, 4.7 yellow and 4.1 white units).

Measurement of the burst strength of different zein films

The burst strength of different zein films were analysed in texture analyser and the results of this study are shown in table 2. The texture analyser plot obtained for different zein films combinations. The peak force shown was burst strength and the time shown against peak force was the time taken to burst, which is the indication of flexibility of the different zein films. The mean burst strength of sample zein+ethanol was highest as compared to zein+ethanol fructose and zein+ ethanol+glycerol combination. In case of distance to burst analysis, zein+ethanol+fructose based zein films showed high value than that of zein + ethanol and zein + ethanol + glycerol combination films respectively.

FTIR analysis of different zein films

The results of FTIR analysis of different combination of zein films prepared are shown in fig. 2. The FTIR spectra of pure zein film exhibited main characteristic bands between 2935.0 and 2965.0 cm^{-1} for stretching of –COO– groups, at 3300.0 cm^{-1} for stretching of –N–H of amines and 1555.0 cm^{-1} for aromatic bending of C=C.

Coating of various model foods using zein films

After the physiochemical characterization, different zein films were used for coating of various model foods *viz*. Himalayan fig (*Ficus palmata*) leather, roasted almond nuts, chocolate cookies and vanilla cookies. The different zein films coating were first done on Himalayan fig leather and results were compared with standard uncoated samples (Fig. 3). The best results of coating were observed with zein (10%) + ethanol (90%) + glycerol (5%) as plasticizer combination based films as compared to pure zein films. The films prepared with gycerol as plasticizer were more flexible, stretchable, opaque and less brittle than other zein films. The similar zein films coating were done on roasted almond nuts, chocolate and vanilla cookies and results are shown in fig. 3. In all these three model foods, the best coating results were observed with zein (10%) + ethanol (90%) + glycerol (5%) as a plasticizer as compared to other zein films. The coating done with Zein (10%) + Ethanol (90%) + Fructose (4%) as plasticizers were also stable than the Zein (10%) + Ethanol (90%) combination films.

Discussion

Films are usually prepared by casting zein solutions on appropriate substrates (Genaddios *et al*., 1994). According to Shukla and Cheryan (2001), Zein films are transparent, glossy, grease proof, tasteless and resistant to microbial attack. Wang *et al*., (2008) showed that smooth, thin and transparent zein films were formed (1 mg/ml in 70% ethanol at pH 3.4) by the addition of chloroacetic acid. Entwistle and Rowe, (1978) described the improvement of flexibility of zein films, if plasticizers are added which acts as a lubricant between molecular chains. Park and Chinnan, (1995) evaluated various physiochemical properties of zein films. It was presumed that the interaction between chloroacetic acid and zein through hydrogen bonds promoted the formation of films.

Conclusion

In present study, different types of zein films were prepared and the physiochemical properties (thickness, colour, solubility and water vapour transmission rate) were evaluated for pure zein films and zein films with combinations of plasticizers. In zein films, highest thickness of 0.22±0.04 mm was observed in zein + ethanol + glycerol film and lowest thickness of 0.14±0.02 mm was observed in zein + ethanol film. The highest solubility (25±0.42%) was observed in zein + ethanol + fructose films and lowest solubility (17±0.67%) was shown in zein + ethanol films. These zein films were used for different model foods to evaluate them as an alternative to commercial coated films which consist of expensive and non-degradable polymers. The best results of coating were observed with zein (10%) + ethanol (90%) + glycerol (5%) as plasticizer combination films as compared to pure zein films. The films prepared with plasticizer gycerol were more flexible, stretchable, less clear and less brittle than other zein films. This zein film can be used for coating on more model food materials or can be explored for use in biodegradable food packaging.

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