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Effect of rice husk silica nanoparticles on physical and mechanical properties of soy protein isolate packaging films

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Abstract
Biosynthesis of silica nanoparticles (SNPs) from rice husk is cost effective, non-toxic and eco-friendly method. The present study was carried out to determine the effect of different concentrations of biosynthesized amorphous SNPs (93.14 nm) on physical and mechanical properties of soy protein isolate packaging films prepared by casting method. The results showed that the moisture content, total soluble matter and water absorption of prepared films decreased with increasing in concentrations of SNPs. The tensile strength increased however, elongation at break decreased with addition of SNPs. There were non-significant effects on thickness, opacity and colour characteristics of developed biodegradable soy protein isolate films. Thus, biosynthesized silica nanoparticles could be used to improve the physical and mechanical properties of biodegradable soy protein isolate packaging films to overcome the defects which have limited the application in food packaging.

Keywords: Rice husk, silica nanoparticles, soy protein isolate

Introduction
Today, the production and use of plastics or non-biodegradable materials as food packaging material have significantly increased. These types of packaging material are usually derived from petroleum products, which causes the problem in waste disposal (Avella *et al.* 2005) [3]. To meet the increasing demand of packaging material for sustainability and environmental safety, researcher are trying to develop food packaging materials that could rapidly degrade and completely mineralize in environment (Jayaramudu *et al.* 2013; Majeed *et al.* 2013) [8, 11]. Biodegradability of biopolymers makes it as favorable alternatives to be exploited and developed into eco-friendly food packaging materials (Tang *et al.* 2012) [28]. Soy protein isolate (SPI) is a complex mixture of proteins with widely different molecular properties (Sabina *et al.* 2013) [21]. Soy protein isolate (SPI) has a significant application potential in the food industry, agriculture, bioscience and biotechnology due to its reproducibility, good biocompatibility, biodegradability and process ability (Song *et al.* 2011) [22]. Biopolymers produced from various natural resources, such as cellulose, starch, polysaccharides, chitosan and various proteins from plant and animal origins, have been considered as attractive alternatives for non-biodegradable petroleum-based plastic packaging materials, since they are environmentally friendly, abundant, inexpensive, renewable, biocompatible and biodegradable (Sorrentino *et al.* 2007; Luckachan and Pillai 2011; Tang *et al.* 2012) [23, 10, 28]. At low to intermediate relative humidity conditions carbohydrate or protein-based packaging films are generally good barriers against oxygen. However, their barrier against water vapor is poor due to their hydrophilic nature. In addition, many biopolymers are relatively sensitive to water, with some materials dissolving rapidly or exhibiting a substantial decrease in mechanical properties when they absorb moisture. The high sensitivity to moisture and poor mechanical properties makes biopolymers limited to the commercial use in food packaging (Cabedo *et al.* 2006; Sorrentino *et al.* 2007; Pan *et al.* 2014; Othman, 2014) [23, 15, 14]. Development of packaging materials and technologies have been carried out over the centuries, but today's demands are looking for a new improvement and innovation (Bradley *et al.* 2011). Diverse methods have been applied by previous researchers such as use of the nanoparticles to improve both the mechanical and moisture resistance properties of biodegradable packaging materials (Abdollahi *et al.* 2012; Nafchi *et al.* 2013; Arfat *et al.* 2014; Han and Wang 2016) [1, 29, 2, 6].

Silica nanoparticles possess specific advantageous properties, which is considered as most useful nanofillers in preparation of nanocomposites. The Silane coupling agent have been used to improve the physical and mechanical properties of composites by facilitating adhesion of inorganic filler particles to the polymer matrices (Stojanovic *et al.* 2009) [24]. Tang *et al.* (2008) [27] developed starch/PVOH/ SNPs based biodegradable packaging films and documented that the water resistance and tensile properties of films enhanced with an increase in their SNPs content also resulted in improved miscibility and compatibility between the film components. According to Avella *et al.* (2005) [3] SNPs have been proved to be a promising option in order to improve mechanical and barrier properties for making nanocomposite.

Every year around 24 million tonnes of rice husk (RH) produced by India. Due to low density and less commercial interest of RH, its handling as well as transportation is problematic and which creates disposal and serious environmental problems (Pode 2016) [16]. The synthesis of amorphous SNPs from plant biomass yields high quality, eco-friendly and cost effective product as opposed to the high energy processing of the inorganics (Liou and Yang 2011) [9]. Therefore, the use of RH as source of SNPs has both positive environmental and economic impact through the use of an abundant low-value agricultural by-product which can be alleviating waste disposal problems. In this sense, biosynthesis of SNPs from RH and its effect on physical and mechanical properties of biodegradable soy protein isolate films was determined.

Materials and methods

Biosynthesis and characterization of silica nanoparticles

Silica nanoparticles were synthesized from rice husk at the optimized conditions given by Rafiee *et al.* (2012) [18]. Biosynthesized SNPs characterized by using Zetasizer (Malvern, ZETA Sizernano 383 issue 5.0, England), fourier transform infrared (Shimadzu, FTIR-8400S, Japan spectra was obtained on a Vertex 70 spectrometer 97 equipped with a digital detector *via* the conventional potassium bromide (KBr) pellet method, X-ray diffraction (Theta-theta type X ray diffractometer, Rigaku corporation, Japan) and scanning electron microscope (Carl Zeiss Microscopy, EVO 10, Germany).

Preparation of soy protein isolate (SPI) based films

Soy protein isolate based packing films were prepared by casting method. Film forming solutions were prepared by mixing 5 g of soya protein isolate (SPI), 20% (wt basis) of sorbitol and 10% (wt basis) of glycerol in 100 ml of distilled water. The different concentration of biosynthesized SNPs (0, 25, 50, 75, 100, 125, 150, 175 and 200 ppm) were added in prepared solutions. The solution was homogenized at 10000 rpm for 2 min. The pH of film forming solution was adjusted to 10 by addition of NaOH. The prepared solution heated in water bath at 90 ± 2 ºC for 30 min and then rapidly cooled in an ice bath for 10-15 min to avoid further denaturation. The solution was filtered through two layers of muslin cloth to remove any coagulation. Filtrated was homogenized at a speed of 10000 rpm for 2 min. The solution poured on Teflon layered wooden sheet frame to form uniform packaging film. Then it was dried at 55 ± 1 ºC for 24 h in try dryer (Han and Wang 2016) [6]. The dried and uniformed films were peeled and measured different physical and mechanical properties.

Physical and mechanical properties of developed biodegradable packaging films

Thickness

The thickness of the developed packaging film was measured with the help of a digital screw gauge with ± 0.001 mm sensitivity. Three measurements at different points were recorded on each film and the average thickness of the films were recorded (Cao *et al.* 2007) [4].

Moisture content

Three 4 cm^2 specimens of each sample were weighed (M_0), dried in hot air oven at 105 ºC for 24 h and weighed (M_d). Moisture content was determined as described by Han and Wang (2016) [6].

$$\text{Moisture content (\%)} = \frac{(M_o - M_d)}{M_d} \times 100$$

Total soluble matter

The dried packaging film samples directly immersed in 30 ml of distilled water and stored at 25 ºC for 24 h and then dried in hot air oven at 105 ºC for 24 h and weighed (M_f). Total soluble matter **(**TSM) was determined by the following formula given by Tajik *et al.* (2013) [25].

$$\text{Total soluble matter (\%)} = \frac{(M_o - M_f)}{M_d} \times 100$$

Water absorption

The developed packaging films were cut into 80 mm × 80 mm size and weighed (W_0) and immersed in 25 ºC water for 10 min. Then the wet samples were wiped with filter paper to remove surface moisture and immediately weighed (W_1). The water absorption (Wa) was calculated using the following equation given by Tang *et al.* (2009) [26].

$$\text{Water absorption (\%)} = \frac{(W_1 - W_0)}{W_0} \times 100$$

Opacity

Developed film specimens were cut into a rectangular shape piece and placed in a spectrophotometer test cell directly and air was used as the reference. A spectrum of each film was recorded on an UV-Visible spectrophotometer (Perkin Elmer, Lamda 35, Germany).The area under the absorption curve from 400 to 800 nm was recorded. The opacity of films was calculated by the following equation described by Cao *et al.* (2007) [4].

$$\text{Opacity (\%)} = \frac{A_{500}}{T} \times 100$$

Where, A_{500}: Absorption of packaging film at 500 nm; T: Thickness of packaging film (mm)

Tensile strength and elongation at break

Tensile strength (TS) of developed packaging film was recorded by using a texture analyzer TA-XT-plus (Stable Micro System). The 50 N load cell equipped with tensile grips used to measure the TS of different packaging films. The tensile strength and elongation at break was calculated by using following formula given by Qing *et al.* (2009) [17].

$$\text{Tensile strength (MPa)} = \frac{F}{L \times W} \times 100$$

Where, F: Tension at break of film sample (N); L: Length of film sample (mm) and W: Width of film sample (mm)

$$\text{Elongation at break (\%)} = \frac{l_b}{l_o} \times 100$$

Where, l_b: Measured elongation at break (mm); l_o: Original specimen length (mm)

Colour

Hunter lab colourimeter (Hunter Associates Laboratory Inc., Colour Flex EZ, United States) used for the measurement of colour of the developed packaging film.

Statistical analysis

The obtained data were analyzed by using completely randomized design. The results were presented as means±Standard Error (SE). Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) method were used to exam the means between treatments and then statistically significant differences between means by using SPSS software version 16.0 at $P \leq 0.01$.

Result and Discussion

Characterization of silica nanoparticles

Zetasizer revealed an average particle diameter of SNPs was 93.14 nm (Fig.1). FT-IR spectra of SNPs as shown Fig. 2 shown that, the broad band between 2800 and 3750 cm^{-1} was due to silinol OH groups and adsorbed water and 2100 to 2300 m^{-1} was due to silane group Si-H. The predominant absorbance peak at 1320 cm^{-1} was due to siloxane bonds (Si-OSi), the peaks between 1200 and 700 cm^{-1} were attributed to vibration modes of the gel network. IR spectrum was clearly shown the pure silica. X-ray diffraction pattern showed broad halo band of absorbance at about 2θ = 15-25 ° region which confirms the amorphous nature (Fig. 3). SEM image revealed that uniformly distributed SNPs were in the agglomerated form with spherical morphology (Fig. 4).

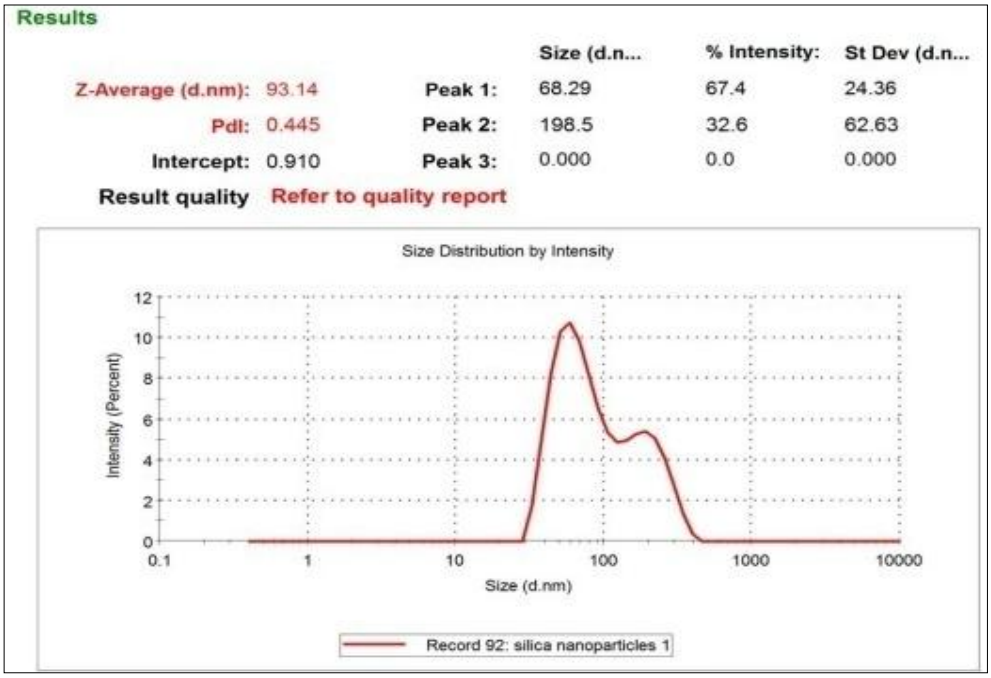

Fig 1: Average particle diameter of silica nanoparticles

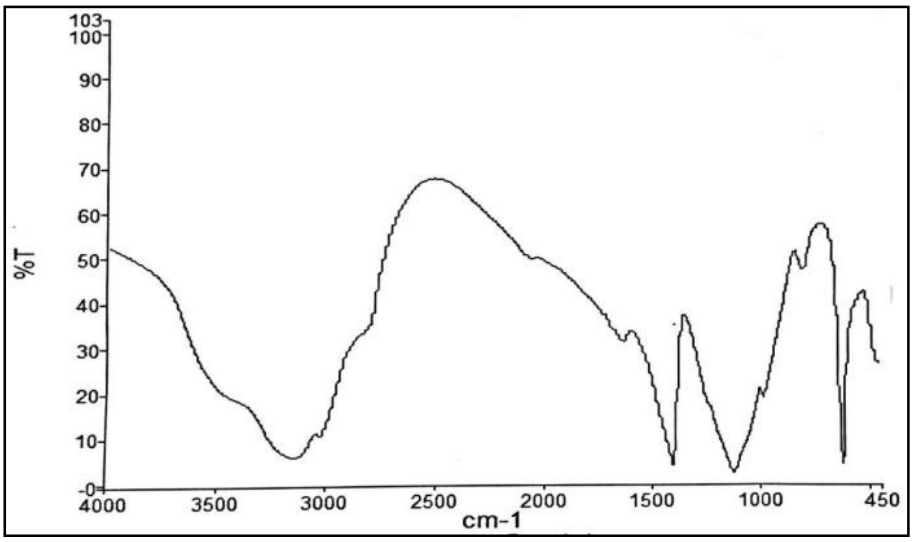

Fig 2: FT-IR spectra of silica nanoparticles

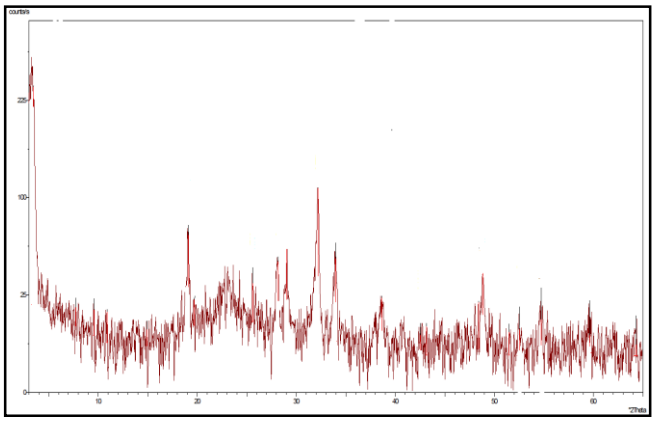

Fig 3: XRD pattern of silica nanoparticles

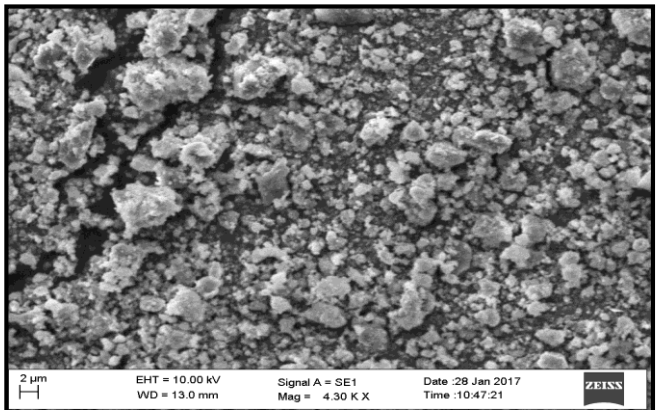

Fig 4: SEM image of silica nanoparticles

Physical and mechanical properties of developed biodegradable packaging films

The thickness of developed packaging films non-significantly affected by concentration of SNPs. This is probably due to the incorporation of SNPs occupy the sites on SPI that normally would be occupied by water (Zou and Yoshida, 2010) [33]. The characteristic of water susceptibility of protein films, which is due to the hydrophilic nature of those macromolecules, could be considered as one of the most disadvantageous properties with respect to certain applications. Thus, an increased resistance to water in biopolymers represent one of the most sought after modifications for their more widespread practical use (Echeverria *et al.* 2014) [5]. The per cent moisture content (MC) of developed packaging films decreased from 23.63 ± 0.16 to 15.80 ± 0.16% as increased in concentrations of SNPs as shown in Table 1. The moisture content reduced by 46.60% over the control at 200 ppm. The amount of water present in composite films is an indication of the films hydrophilicity, the more hydrophilic films being those that present the highest values of moisture content. According to Han and Wang (2016) [6], SNPs were ascribed to getting trapped inside the SPI matrix, restricting the entry of water molecules which resulted in decreased moisture content. Similar result for nanobiocomposites were reported by Nafchi *et al.* (2013) [12] incorporation of nanoclay and nanosilica in starch (Han and Wang (2016)) [6], SNPs in SIP matrix and nano kaolin into semolina films (Jafarzadeh *et al.* 2016) [7].

The total soluble matter (TSM) of the films is usually measured to assess water resistance and product integrity. Solubility in water is a major property of protein films that is related to its structural properties as well as the presence of components in the films, since potential applications may require water insolubility to enhance the product integrity and water resistance (Jafarzadeh *et al.* 2016) [7]. TSM of developed packaging films was decreased with increased in concentrations of SNPs. The concentrations 200 and 175 ppm showed 14.15% and 12.10% decreased of TSM over the control respectively. This might be due to the formation of strong hydrogen bonds between the SPI matrix and SNPs, which created a relatively integrated structure of film and reduced their sensitivity to water. Finally, incorporation of SNPs suppressed the diffusion of water into the structure (Han and Wang 2016) [6].

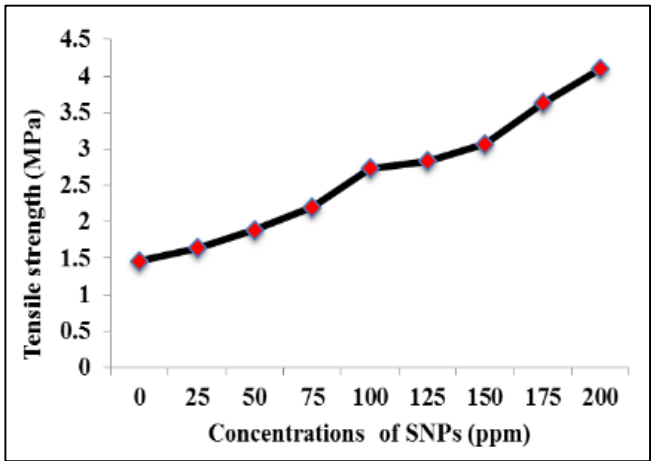

Fig 5: Effect of silica nanoparticles on tensile strength of biodegradable packaging films

The results of the present study are in good agreement with previous work by Voon *et al.* (2010), Torabi and Nafchi (2013) [13] and Han and Wang (2016) [6] who reported as decrease in the water solubility due to the loading of SNPs to bovine gelatine films starch, potato starch and SPI matrix, respectively.

Table 1: Physical and Mechanical Properties of Developed Biodegradable Packaging Film

SNPs Concentrations	Thickness (mm)	Moisture content (%)	Total soluble matter (TSM) (%)	Water absorption (%)	Opacity (%)	L* value	a* value	b* value
0	0.211	24.63^{i}	34.93^{h}	80.10^{a}	482.00	32.80	-0.90	10.64
25	0.212	23.63^{h}	34.20^{g}	79.00^{c}	482.00	32.73	-0.99	10.65
50	0.211	22.00^{g}	33.73^{ef}	78.47^{cd}	481.77	32.67	-1.11	10.65
75	0.213	21.43^{f}	33.60^{e}	77.90^{d}	481.60	32.60	-1.19	10.66
100	0.213	19.90^{e}	33.00^{d}	76.00e	481.60	32.53	-1.21	10.66
125	0.212	19.00^{d}	32.30^{c}	75.70^{ef}	481.80	32.52	-1.24	10.67
150	0.213	18.20^{c}	32.06^{c}	75.00^{fg}	481.60	32.47	-1.27	10.67
175	0.212	17.40^{b}	31.16^{b}	74.60^{bg}	481.80	32.43	-1.31	10.68
200	0.213	16.80^{a}	30.60^{a}	74.00^{b}	481.60	32.25	-1.32	10.69

Similar results also reported for methyl cellulose/ montmorillonite nanocomposite films (Tunc and Duman 2010) [30]. Low water solubility is extremely important since such materials could be more resistant in wet conditions when used as coatings (Rhim *et al.* 2005) [20]. As one of the major drawbacks in the use of a protein based packaging material is its water absorption tendency, any improvement in water resistance is therefore highly important. The per cent water absorption was decreased with an increased of concentration of SNPs. The significantly 8.20% and 7.37% water absorption decreased in concentrations of 200 and 175 ppm over the control. This phenomenon can be described by the interactions between SNPs and other compounds in biopolymer film structure.

According to Tunc and Duman (2010) [30] when the SNPs content of films was increased, more hydrogen bonds formed between the silica and the matrix components. For this reason, free water molecules did not interact as strongly as with SPI/SNPs films as compare with SPI films alone. Similar results were recorded by Tang *et al.* (2009) [26] and Torabi and Nafchi (2013) [29] as decreased in water absorption due to the loading of SNPs to starch film due to network structure formed by combining of SNPs with starch/PVA, which prevented the water molecules from dissolving the SNPs film and improved the water resistance of the film. The per cent opacity of developed biodegradable packaging films prepared under different concentrations of SNPs was mainly depends on the thickness of packaging films. Which is non-significantly differ by SNPs. This might be due to non-significance of thickness and closed refractive indices between the SNPs (1.47) and SPI in NaOH solution (1.45).Our results are in good agreement to previous work by Wen *et al.* (2009) [32] who recorded the excellent optical transparency of the nano-composites with independent of the SNPs concentrations for Poly (L-lactide) (PLA)/silica (SiO_2) nanocomposites.

The mechanical properties involves scientific, technological and practical aspects because polymer materials may be subjected to various kinds of stress in the process of usage. According to Jafarzadeh *et al.* (2016) [7] increment in tensile strength (TS) is a vital criteria in food packaging applications, because high TS can allow packaging films to withstand ordinary stresses encountered during food handling, delivery and transportation. The mechanical properties of nanocomposite films have been reported to be strongly dependent on the interfacial interaction between the matrix and filler. The mean TS values of SPI films composted with SNPs were increased from 1.64 to 4.10 MPa as shown in Fig. 5. TS increased by 64.39% and 59.79% in 200 ppm and 175 ppm concentration over the control respectively. According to Rhim *et al.* (2005) [20], the increased concentration of SNPs counterparts increases the interaction between SNPs and SPI matrix which can restrict the molecular motion of the polymer chains and enhance the structure (Han and Wang 2016) [6]. The percentage elongation at break (E), a measure of the extensibility of films, generally shows an inverse relationship with TS, *i.e.,* it decreases as TS increases. Meanwhile, a decreasing trend was observed for elongation at break. Reduction in EB can be advantageous in food packaging, as this property is directly related to the bio-degradability of the films (Voon *et al.* 2012) [31]. The per cent elongation at break decreased as concentrations of SNPs increased. The lower per cent elongation at break observed in 200 ppm, which was lower than 11.97% over the control as shown in Fig. 6. According to Tang *et al.* (2009) [26] water has a plasticizing role on the starch and addition of SNPs decrease the water content and consequently reduced the elasticity (elongation at break) of the films. The Pearson's correlation coefficients showed that positive linear relationship (r = 0.99) between moisture content and elongation at break also there was a negative linear relationship (r = -0.97) between moisture content and tensile strength. Increased moisture content was increased in flexibility of the film. The positive linear relationship between moisture content and elongation at break also negative linear relationship between moisture content and tensile strength same were result was recorded by Nafchi and Alias (2013) [12]. The surface colour of food packaging films is an important parameter because of its influence on appearance and consumer acceptance. There was non-significant difference between the control and all treatments of colour of developed packaging films.

Conclusion

Addition of biosynthesized silica nanoparticles showed significantly improved the physical and mechanical properties of the soy protein isolate biodegradable packaging films. The addition of 200 ppm of silica nanoparticles had good potential to improve mechanical strength *viz.,* water absorption (74.60%) tensile strength (3.63 MPa) and per cent elongation at break (107.27%) for overcoming the defects which have limited the application of soy protein films in food packaging. In future, a systematic study on biodegradable packaging film with different storage conditions with addition of biosynthesized silica nanoparticles can be carried out.

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