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Chitosan and Starch Based Intelligent Films with Anthocyanins from Eggplant to Monitor pH Variations[#]

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The objective of this study was to develop and characterize a pH indicator based on chitosan (C) and starch (S) including anthocyanins from eggplant to indicate food quality changes through the detection of changes in the pH of packaged food products. Anthocyanins were extracted with solvents including ethanol and water (7:3, V:V) (EgP-E) and water (EgP-W) in acidic pH. The pH indicator films were obtained incorporating anthocyanin as 1.5 g extract/100 g film solution. The optical, mechanical and water vapor permeability properties were used to characterize the pH indicator films. The total monomeric anthocyanin content and phenolic content of extract solutions were also determined. Color variations of pH indicator films were measured by a colorimeter after immersion in different pH buffers (pH 2.0–10.0). Initially, dried C films and S based films were observed in a violet color and a magenta color, respectively. C and S films with anthocyanins showed color variations from pink (in acidic pH) to bluish-green (in neutral pH) and to violet (in basic pH) in different pH values. The water vapor permeability of films with anthocyanins was higher than films without anthocyanins. Additionally, C and S based films including anthocyanins extracted with water showed better permeability values. C:EgP-W and S:EgP-W films presented higher elasticity values when compared with films including ethanolic extracts ($p<0.05$) due to the plasticizing effect of water. As expected, opacity values of films including both E and W extracts were higher than those obtained in films without extracts. The developed pH indicator films have potentials to be used as a diagnostic tool for the detection of food spoilage with the advantages of a simple manufacturing process, biodegradability, and usage of natural and safe compounds.

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Introduction

Bio-based polymers are considered as environmentally friendly because they are more easily decomposed in nature than commonly used plastics. Therefore, there is an increasing interest in the development of packaging materials including natural polymers due to their biodegradability and barrier ability against to the transport of moisture, gas, aroma, and lipids (Dutta et al., 2009; Yoshida et al., 2010). In addition, the properties of those bio-based polymers can be changed by the addition of substances such as colorants, antimicrobials, plasticizers, etc. It is quite a new issue that packaging materials obtained from natural polymers are converted into intelligent packaging systems by the addition of different components (Yoshida et al., 2014).

Intelligent packaging is a system providing information on shelf-life, safety and quality of food product by monitoring the changes inside the package (Yam et al., 2005). Among the intelligent packaging systems, color-based pH indicators have promising potential use as indicators of oxidation, microbial growth or microbial metabolites (Smolander, 2003; Kerry et al, 2006). Many factors, such as water activity, pH and oxygen can affect the shelf life of the product. The pH levels of food products may vary depending on factors such as flavor, consistency and the formation of chemical reactions that may affect shelf life. The food spoilage is usually accompanied by a pH change, thus the relationship between the food product quality and the changes in pH is very strong (Veiga-Santos et al., 2011). The correlation between the packaged food product and the changes in pH during the storage is of great importance. Thus, a smart system that allows the consumer to evaluate the quality of food products with changes in the color of packaging material stemmed from pH is of the innovative approaches. There is a growing interest on bio-based pH indicator systems such as cassava starch (Veiga-Santos et al., 2011), chitosan (Kato et al., 2011; Maciel et al., 2012; Maciel et al., 2015), cornstarch (Silva-Pereira et al., 2015), gum/cellulose matrix (Ma et al., 2017).

Anthocyanins are polyphenolic plant pigments, which show different colors depending on many factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents and the presence of enzymes, proteins and metallic ions (Castañeda-Ovando et al., 2009).

There are few studies related to pH indicators based on, chitosan and starch film including anthocyanins, which undergo color modification when exposed to different pH environments. Thus, in this study, a bio-based intelligent packaging material film with eggplant extracts, as sources of pH indicators (anthocyanin), incorporated into starch and chitosan matrices were developed. The mechanical properties, water vapor barrier, optical properties of the films were evaluated as well as the color change at different pH values.

Materials and Methods

Materials

Chitosan (C) (ChitoClear® CG1600, high molecular weight>320 kDa, 1600 Cps solution at 2% with acetic acid,>75% deacetylated) was purchased from Primex (ChitoClear®, Siglufjordur, Iceland) and starch (S) was supplied from Sigma-Aldrich (St. Louis, Missouri, USA). All chemicals were analytical grade and purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Eggplants were provided from a local market in Isparta/Turkey and stored under suitable conditions prior to extraction.

Extraction of Anthocyanins

Anthocyanins were extracted from eggplant peels (EgP) according to the method described by Choi et al., (2017) with some modifications. Briefly, 30 g of EgP was exposed to 100 ml of selected solvent at pH 2 adjusted by an acetic acid solution. Two kinds of solvents were prepared with water (W) and water:ethanol (E) (3:7, v:v). The extraction was performed under room temperature at shaking stirrer during 24 h followed by ultrasonication for 30 min. All extracts were then filtered under vacuum and concentrated by an evaporator at 40°C. The concentrated solutions were stored at -18°C prior to their use.

Total Phenolic Content and Anthocyanin Concentration of Extracts

The total anthocyanin content of extracts was determined by the pH-differential method (Giusti and Wrolstad, 2001). Briefly, the pH of the extracted solution (1 mL) was one adjusted with potassium chloride buffer (10 mL), pH 1.0, and the other with sodium acetate buffer (10 mL), pH 4.5. After 15 min, the absorbance of each dilution was recorded at the 510 and 700 nm against a blank cell filled with distilled water. The absorbance of extracts (A) was corrected as follows:

$$A = (A510\text{-}A700)_{pH\ 1.0}\text{-}(A510\text{-}A700)_{pH\ 4.5}$$

The monomeric anthocyanin concentration in the original sample was calculated using the following formula:

$$\text{Monomeric anthocyanin} = (A \times MW \times DF \times 1000)/(\varepsilon x 1)$$

Where MW is the molecular weight (449.2 g/mol for cyanidin-3-glucoside), DF is the dilution factor, and ε is the molar absorptivity (26,900 for cyanidin-3-glucoside).

Total phenolic content of extracts was determined by the Folin-Ciocalteu colorimetric method (Singleton et al., 1999). The suitable dilutions of all extracts were mixed with Folin-Ciocalteu reagent (Sigma-Aldrich, Missouri, USA) (0.2 N) and sodium carbonate (7.5% w/v), and measured by reading the absorbance of samples at a wavelength of 765 nm using a UV-Visible spectrophotometer (Shimadzu, UV-1601, Tokyo, Japan). The results were expressed as mg gallic acid equivalent per kg sample.

Film Preparation

Chitosan (C) at 1% w/w was dissolved in 1% w/w aqueous acetic acid solution. A C film solution was obtained by adding glycerol at 0.20% w/w (Sanchez-Gonzalez et al., 2010). The starch (S) powder was dissolved in distilled water (2%, w/w) and heated at 80°C for 15 min. Then, the film-forming solution was further stirred for 25 min. Glycerol was added to the fully dissolved solutions at 0.40% w/w. Both water (W) and water:ethanol (3:7, v:v) (E) based EgP extracts at 5% v/v in a film solution, were then added to the film solutions and homogenized (DAIHAN HG-15A, Korea) for 5 min. After degassing to avoid the formation of air bubbles, 50 g of the solution was cast onto a Teflon® coated plate (Ø=150 mm) and dried in ambient conditions for 48 h. All film samples were conditioned at 25°C and 50% RH for one week before analysis.

Mechanical Properties of Film Samples

Elastic modulus (EM), tensile strength (TS) and elongation (E, %) at break point values were determined by the ASTM standard method D882 (ASTM, 2018). Films (2.5 cm×5 cm) were mounted in the film-extension grips of the testing machine (Lloyd LR5, AMETEK, Inc, West Sussex, UK) and stretched at 50 mm/min until they broke.

Water vapor permeability and optical properties of film samples

The water vapor permeability (WVP) of film samples were determined according to E96-95 gravimetric method (ASTM, 2016). All film samples were exposed to 100% RH, and the permeability measurements were performed by weighing the cups periodically (every 1.5 h for 48 h) at 25°C.

Transparency (T, %) of the films was determined by measuring the percent transmittance at 450 nm using a UV–vis spectrophotometer (Shimadzu, UV-1601, Tokyo, Japan). Rectangular strips of film samples (1×4 cm) were obtained to determine the opacity. All films were placed in a UV–vis spectrophotometer test cell, and the absorption spectrum of the sample was obtained from 400 to 800 nm (Shimadzu, UV-1601, Tokyo, Japan). Film opacity was measured as the area under the curve divided by the film thickness and expressed as absorbance unit (AU nm/mm).

Color Response of Film Samples at Different pH Values

The color changes of the films at different pH values (2-10) were measured with a Minolta Chroma Meter (CR-400, Konica Minolta, Inc., Japan) by immersing the film samples in different pH buffers. A white standard calibration plate (Y=92.7, x=0.3160, y=0.3321) was used as a background for the color measurement of the films. All

measurements were taken at three random locations and results were expressed as CIE *L**, *a** and *b**. Tests were carried out in triplicate.

Statistical Analysis

An analysis of variance (ANOVA) and Tukey's multiple comparison tests was used to compare the different treatments at a 95% confidence level (Minitab 17 software, Minitab Inc., Brandon, UK). Three observations (at least) were performed for each film, and each experiment was replicated three times.

Results and Discussion

Total Anthocyanin and Phenolic Content of Egp Extracts

Total monomeric anthocyanin content and phenolic concentration are shown in Table 1. The total monomeric anthocyanin content ranged from 8.7 to 49.7 mg per 100 g, which is similar to the results obtained by Boulekbache-Makhlouf et al. (2013). The ethanolic extracts showed higher monomeric anthocyanin content, probably due to better extraction in ethanol when used acetic acid solution.

The total phenolic content values obtained for ethanolic extracts were significantly higher than those obtained for the water extracts were. Similar results were presented by Jung et al. (2011), who evaluated the phenolic content of different parts (calyx, leaf, peel, pulp, and stem) of eggplant after extracted with 70% ethanol and water. In contrast, the anthocyanin content of eggplant extracts obtained in this study was different from reported by Azuma et al. (2008), which might be due to the differences in the growth condition, variety, and cultivation.

Film Characterization

Mechanical properties of film samples: The EM, TS, and E (%) values of film samples are shown in Table 2. C:EgP-E films exhibited the highest EM and TS values while S films exhibited the lowest values ($P<0.05$). The addition of both extract type into film samples enhanced the mechanical properties of films suggesting that there was an intermolecular crosslink within the matrices of the films (Coughlan et al., 2004). Similarly, Yong et al. (2019) reported that chitosan films including eggplant extracts had higher TS values when compared to neat chitosan films. The enhancement in EM and TS might be probably due to the hydrogen bonding between polymer and phenols in the extracts (Koosha and Hamedi, 2019). C-based films showed significantly higher EM, TS and E values ($P<0.05$), which could be explained by the stronger interaction between hydroxyl/amino groups of C and polyphenols (Yong et al. 2019).

Film samples including W-extracts showed slightly higher elongation capacities probably due to the plasticizing effect of water ($P<0.05$). Besides, film samples including ethanolic extracts presented lower elongation values ($P<0.05$). Ethanolic extracts might cause an anti-plasticizing effect leading a decrease in flexibility by limiting the motion of polymer chains (Mushtaq et al. 2018). This observation is in agreement with Gutierrez and Alvarez (2018) who studied cornstarch films including natural and modified nano-clays with or without added blueberry extract.

WVP and optical properties of film samples: WVP and optical properties of film samples are shown in Table 3. S-based films showed higher thickness values when compared to C-based films. The thickness of films decreased when extracts were added to C-based films, whereas the thickness of S-based films including extracts presented higher values ($P<0.05$). The interactions between the anthocyanins and film matrix might affect the thickness of film samples. Similar results were reported for chitosan films including natural extracts (cranberry, blueberry, purple sweet potato) by Lozano-Navarro et al. (2017).

Table 1 The anthocyanin and phenolic content of extracts

Extract	Total phenolic content (mg GAE/kg)	Total monomeric anthocyanin content (mg/100 g)
EgP-E	1573.7±1.4^{a}	49.7±1.1^{a}
EgP-W	275.2±8.5^{b}	8.7±0.1^{b}

$^{a-b}$ Means in the same column with different superscripts differ significantly ($p<0.05$) according to Tukey test.

Table 2. Mechanical properties of film samples

Film samples	EM (MPa)	TS (MPa)	E (%)
C	717.27±40.66^{a}	13.57±3.03^{abc}	16.78±1.06^{a}
C:EgP-W	718.34±86.19^{a}	19.23±3.19^{ab}	16.92±2.44^{a}
C:EgP-E	774.02±87.48^{a}	19.29±3.74^{a}	16.38±2.24^{a}
S	153.14±19.76^{b}	2.92±1.47^{bc}	2.65±0.42^{b}
S:EgP-W	185.84±30.81^{b}	5.14±0.88^{bc}	5.21±0.48^{b}
S:EgP-E	204.33±32.54^{b}	5.35±0.77^{c}	1.80±0.33^{b}

$^{a-c}$Means in the same column with different superscripts differ significantly ($P<0.05$) according to Tukey test

Table 3 WVP and optical properties of film samples

Film samples	Thickness (μm)	WVP (g-mm/kPa-h-m^2)	Opacity (AU nm/mm)x10^3	T (%)
C	40.00±6.24^{bc}	14.31±1.56^{b}	1.75±0.21^{b}	58.60±2.36^{a}
C:EgP-W	39.67±2.08^{bc}	11.30±3.46^{b}	2.19±0.58^{a}	55.15±1.40^{a}
C:EgP-E	32.33±0.58^{c}	10.11±1.68^{bc}	3.85±0.35^{ab}	50.65±1.45^{a}
S	47.00±1.00^{ab}	15.28±0.39^{c}	1.98±0.05^{b}	40.90±2.59^{a}
S:EgP-W	50.67±3.06^{a}	11.71±1.94^{bc}	2.03±0.57^{b}	35.15±0.39^{a}
S:EgP-E	53.67±1.15^{a}	37.92±1.77^{a}	2.08±0.60^{ab}	37.90±3.19^{a}

$^{a-c}$ Means in the same column with different superscripts differ significantly ($P<0.05$) according to Tukey test

S:EgP-E films showed the highest WVP values, and C:EgP-E films had the lowest WVP values (P<0.05). The presence of EgP extracts in C-based films caused an increase in barrier ability against water. Kurek et al. (2018) reported similar trends for chitosan films including blackberry and blueberry extracts. However, the incorporation of water extracts of EgP into S films decreased the WVP values of the film samples (P<0.05) while adding ethanolic extracts into S films resulted in a significant increase in WVP values. The decrease in WVP values can be explained by the intermolecular interactions between polymer and phenolics leading a decreased affinity towards water molecules (Kurek et al. 2018). Similarly, Yoshida et al. (2014) reported that the addition of anthocyanins into chitosan films reduced WVP values due to the more compact and dense structure of anthocyanin added films when compared to chitosan films without extracts. In contrast, the increase in WVP values of S:EgP-E might be probably due to the high anthocyanin contents presented in ethanolic extracts, causing less dense structure in S-based films (Liu et al. 2018).

All film samples presented an average clearance, thus good transparency was not achieved with all formulations. In general, C and S film samples including extracts showed lower transmittance and higher opacity values, which can be related to the impenetrable matrix light scattering throughout the film (Tongnuanchan et al., 2013). The lower transmittance and higher opacity values are also related to the UV absorption ability of anthocyanins (Peralta et al. 2019).

The opacity values of film samples including ethanolic extracts were found higher than those obtained for films including water extracts (P<0.05). This behavior might be due to the higher anthocyanin contents of ethanolic extracts absorbing more light (Peralta et al. 2019). Lower transmittance values were also reported by other researchers who studied chitosan films incorporated with anthocyanin rich extratcs (Ma et al. 2018; Wang et al. 2019). In a similar manner, Slavutsky et al. (2012) found a decrease in transparency by the addition of nano-clay (montmorillonite) dependent on the dispersion method used.

Color Changes of Film Samples at Different Ph Values

The color changes of S:EgP-W film samples ranging from red to green-blue in pH 2-10 buffers are shown in Figure 1 (other films was not shown here). The incorporation of anthocyanin extracts obtained from EgP helped to develop a bio-based intelligent film (Gutierrez et al., 2017). EgP extracts have the potential to change color at different pH values due to the structural variations occurring within the anthocyanin molecules (Gutierrez and Alvarez, 2018). Film samples started to have a reddish color when exposed to acidic pH levels (2-6) and then films became bluish green at higher pH levels (7-10). Similar results were reported for pH-sensitive film samples including anthocyanin from different plant sources such as purple sweet potato (Choi et al., 2017), blueberry pomace (Luchese et al., 2017), red cabbage (Pereira et al., 2015), black bean (Prietto et al., 2017) and grape skin (Ma and Wang, 2016).

Table 4 also shows the color values of film samples as a function of pH buffers. At higher pH levels, film samples tended to be darker related to the lower *L** values. Film samples immersed in higher pH levels than 5-6, showed significantly lower *L** values (P<0.05). Besides, the *a** and *b** values varied as a function of pH. Higher *a** values were observed in lower pH values (P<0.05). *b** values tended to decrease up to pH 5, and then started to increase at higher pH values (P<0.05). It indicates that more reddish and bluish film samples were related to lower and higher pH values, respectively.

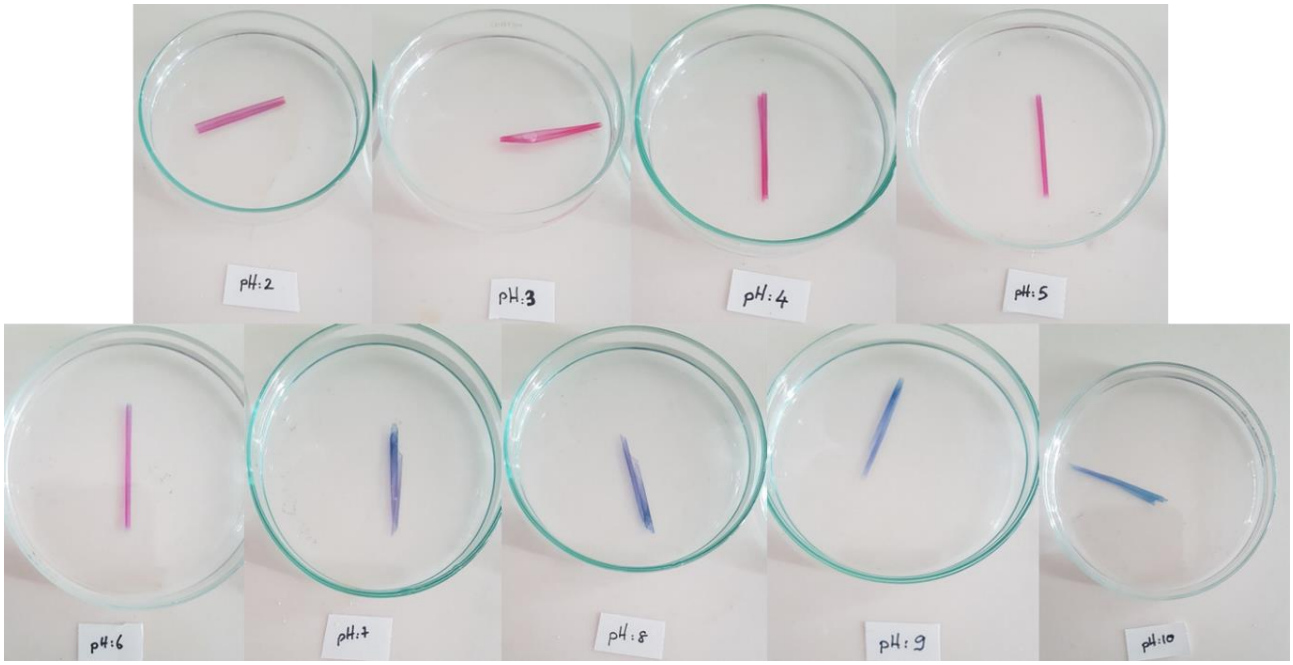

Figure 1 Color response of S:EgP-W film samples in different pH buffers (other films was not shown here)

Table 4 *L**, *a** and *b** color parameters of film samples immersed in different pH buffers (2-10).

Film samples	pH value	Color parameters		
		*L**	*a**	*b**
C:EgP-W	2	86.99±0.39^{a}	-0.58±0.13^{a}	4.36±0.04^{a}
	3	83.60±0.06^{a}	-1.56±0.03^{b}	2.77±0.01^{bc}
	4	86.96±0.02^{a}	-2.32±0.01^{c}	1.99±0.01^{de}
	5	86.98±0.28^{a}	-2.49±0.06^{cd}	2.21±0.01^{cd}
	6	86.08±2.23^{a}	-2.51±0.22^{cd}	2.61±0.52^{bc}
	7	87.79±2.12^{a}	-2.48±0.11^{cd}	2.38±0.25^{bc}
	8	86.44±7.12^{a}	-1.69±0.27^{b}	1.42±0.02^{e}
	9	83.92±0.01^{a}	-3.23±0.01^{e}	3.08±0.01^{b}
	10	85.29±0.08^{a}	-2.94±0.04^{de}	2.91±0.12^{bc}
S:EgP-W	2	89.35±0.07^{a}	-0.18±0.03^{b}	2.59±0.60^{bc}
	3	88.70±0.90^{a}	0.45±0.08^{a}	2.95±0.02^{ab}
	4	86.01±0.11^{a}	-1.26±0.07^{c}	2.15±0.01^{c}
	5	89.70±0.03^{a}	-1.72±0.05^{d}	2.47±0.10^{bc}
	6	86.64±0.64^{a}	0.50±0.01^{a}	2.32±0.01^{c}
	7	83.46±0.37^{a}	-1.79±0.03^{d}	-1.49±0.15^{e}
	8	82.16±0.73^{a}	-3.51±0.06^{f}	-0.79±0.31^{d}
	9	85.70±0.18^{a}	-2.38±0.23^{e}	3.49±0.09^{a}
	10	88.50±6.80^{a}	-1.68±0.16^{d}	3.18±0.20^{a}
C:EgP-E	2	88.09±0.25^{a}	-1.12±0.02^{a}	4.07±0.03^{a}
	3	84.77±0.08^{bc}	-1.31±0.11^{a}	2.30±0.24^{ef}
	4	83.34±0.01^{bc}	-2.47±0.01^{bc}	2.09±0.02^{f}
	5	84.55±2.18^{bc}	-2.27±0.37^{bc}	2.30±0.01^{ef}
	6	85.99±0.15^{ab}	-2.02±0.01^{b}	3.28±0.01^{b}
	7	82.69±0.02^{c}	-2.37±0.01^{bc}	2.79±0.01^{c}
	8	83.16±0.07^{bc}	-2.76±0.01^{c}	2.72±0.01^{cd}
	9	83.96±0.08^{bc}	-2.58±0.04^{c}	2.41±0.01^{de}
	10	83.68±0.01^{bc}	-2.73±0.01^{c}	2.11±0.01^{ef}
S:EgP-E	2	85.34±0.01^{bc}	0.91±0.15^{a}	3.04±0.13^{c}
	3	84.12±0.49^{d}	1.03±0.06^{a}	5.49±0.10^{a}
	4	82.85±0.28^{e}	-2.11±0.03^{e}	0.22±0.05^{g}
	5	88.16±0.01^{a}	-1.46±0.01^{cd}	1.16±0.00^{f}
	6	85.14±0.11^{cd}	-2.26±0.01^{e}	1.76±0.01^{d}
	7	69.53±0.13^{g}	-0.71±0.11^{b}	-0.52±0.01^{h}
	8	85.65±0.35^{b}	-1.32±0.04^{cd}	0.27±0.06^{g}
	9	84.49±0.35^{cd}	-1.49±0.00^{d}	1.46±0.04^{e}
	10	81.16±0.16^{f}	-1.21±0.02^{c}	4.03±0.01^{b}

$^{a-h}$ Means in the same column with different superscripts differ significantly (P<0.05) according to Tukey test

Conclusion

Bio-based (chitosan and starch) pH-sensitive intelligent films were successfully obtained. Film samples including extracts showed lower WVP values except for S:EgP-E films and higher EM and TS values when compared to related control films. The transmittance of film samples did not alter significantly for anthocyanin-included films however, the opacity increased with the incorporation of both type of extracts. Both S- and C-based films including anthocyanins showed a color difference as a function of pH, which was reddish in lower pH and bluish in higher pH levels. Thus, this study showed the potential of incorporating EgP extracts into S and C-based matrices in order to develop intelligent bio-based films giving pH variation information during the storage and handling of a sensitive food product.

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