Development of bionanocomposite packaging film based on lignin nanoencapsulated anthocyanins extracted from agro-waste for enhancing post-harvest shelf life of tomatoes

Maninder Meenu,†[a] Anil Kumar Pujari [b], Seema Kirar [b], Mansi [b], Aniket Thakur [b], Monika Garg †[a] and Jayeeta Bhaumik †[b]

[a] Division of Agricultural Biotechnology, National Agri-Food Biotechnology Institute (NABI), Sector 81, Sahibzada Ajit Singh Nagar, Punjab-140308, India.
[b] Department of Nanomaterial and Application Technology, Center of Innovative and Applied Bioprocessing (CIAB), Sector 81, Sahibzada Ajit Singh Nagar, Punjab-140308, India

†Corresponding author: National Agri-Food Biotechnology Institute, Sector 81, Sahibzada Ajit Singh Nagar, Punjab-140308, India. Tel.: +91 75895 92370; E-mail address: meenu_maninder@yahoo.com.

†Corresponding author: National Agri-Food Biotechnology Institute (NABI), Sector 81, Sahibzada Ajit Singh Nagar, Punjab-140308, India. Tel.: +91 172 522 1205; E-mail address: mkajgarg@gmail.com.

†Corresponding author: Center of Innovative and Applied Bioprocessing (CIAB), Sector 81, Sahibzada Ajit Singh Nagar, Punjab-140308, India. Tel.: +91 172 5221539; E-mail address: jayeeta@ciab.res.in.

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S 1. Characterization of PVA-PEG-A-LNPs packaging films

S 1.1. Thickness

The thickness of bare PVA-PEG and PVA-PEG-A-LNPs films was determined using a digital micrometer (IP65, Mitutoyo, Brazil) with an accuracy of 0.001 mm. The eight different positions of film were measured and the average thickness was calculated for the films.

S 1.2. UV spectroscopy

The UV barrier property of bare PVA-PEG and PVA-PEG-A-LNPs-based packaging films was measured using a UV–Vis spectrophotometer ranging from 200–700 nm. The rectangle film specimens were cut and placed directly in a spectrophotometer test cell. The air was used as a reference. The measurement was repeated thrice for each type of film, and an average was reported as a result.

S 1.3. FTIR

The FTIR spectra of packaging film (5 mm x 5 mm) were recorded with an FTIR spectrometer equipped with an attenuated total reflectance (ATR) accessory (UATR Two, Perkin Elmer, USA). The packaging films (5 mm x 5 mm) were placed on an ATR diamond crystal for spectral collection. A total of 32 scans were collected from 4000 to 400 cm^{-1} with a spectral resolution of 4 cm^{-1} while keeping the sample in contact with the diamond crystal.

S 1.4. SEM

The bare PVA-PEG and PVA-PEG-A-LNPs-based packaging films (5 mm x 5 mm) were gold coated with an ions sputter system (Q150T ES Quorum, UK) for 2 min. Then these gold-coated films were observed under a scanning electron microscope for their morphological exploration (1142265, Thermo Fisher, Czech Republic) at 10 kV.

S 1.5. Tensile strength and elongation at break

The mechanical properties (tensile strength and elongation at break) of bare PVA-PEG and PVA-PEG-A-LNPs-based packaging films were measured using Texture Analyser (TA-HD plus, Stable Micro Systems, UK) by employing a 50 kN load cell equipped with tensile grips. The preconditioned films (70 mm × 20 mm) with 50% relative humidity were placed between the tensile grips with a 40 mm gap and a crosshead speed of 2 mm/s. The experiments were performed in triplicates. The tensile strength and elongation at break were calculated using the following equations.

Tensile strength (MPa) = F/A

Elongation at break (%) = $L_f - L_o/L_o \times 100$

Where F represents force, A indicates the area, L_f is the final length, L_o presents the initial length.

S 1.6. Water Vapor Transmission Rate (WVTR)

To determine WVTR, the silica gel was completely dried at 100 °C for 3 h. The developed bare PVA-PEG and PVA-PEG-A-LNPs-based packaging films have been set on a desiccator with two different relative humidity (RH) media on top of the penetration cup for which water was used for 100% RH, and silica was used for 0% RH. After 24 h, the weight change in packaging films was recorded and data were calculated using the following equation.

WVTR= change in weight/ area exposed film*Time

S 1.7. Migration test of packaging films

The specific migration test of packaging films was performed using four food stimulants namely, water, 3% acetic acid (w/v), 50 % ethanol (v/v) as aqueous simulants, and n-pentane as a fatty simulant. A piece of PVA-PEG and PVA-PEG-15%BB@ALNPs-based packaging films (12 cm^2) and 20 mL of simulant with an area-to-volume ratio of 6 dm^2/1 L were poured into 50 mL glass vials. The samples (packaging films + simulant) were placed in a thermostatic oven at 40 °C for 10 days. Then, samples were removed and simulant was extracted using SPME (for aqueous samples) and n-pentane was concentrated. Three replicates were performed and evaluated for each sample and a homogeneity test was performed for packaging films.

S 2. Quality analysis of tomatoes

The quality parameters of tomato samples in different groups were analyzed for 15 days with an interval of three days as mentioned below.

S 2.1. Firmness

The firmness of tomato samples in different groups was measured using a Texture analyzer (TA-HD plus, Stable Micro Systems, UK). The tomato samples were compressed using a 4 mm cylindrical probe and a 500 N load cell. The firmness of tomato samples was recorded on days 0, 3, 6, 9, 12, and 15, with a pretest speed of 1 mm/s and a test speed of 0.5 mm/s.

S 2.2. Color

The color values (L*, a*, b*) of tomato samples in different groups were measured using a colorimeter (CR 400, Konica Minolta, Japan). Three different readings for color values were taken from three different points on the circumference of the samples. The average of these data was reported as a final result.

S 2.3. Weight loss

The tomato samples were weighed using a precision scale (ME204, METTLER Toledo, USA) at days 0, 3, 6, 9, 12, and 15. The percentage of weight loss on a particular day was determined by employing the following equation.

$$\text{Weight loss (\%)} = W_i - W_f / W_i \times 100$$

Where W_i presents the tomato weight at 0 d and W_f indicates tomato weight at each sampling time.

S 2.4. pH

The tomato samples were ground using a blender to achieve a uniform pulp and filtered using a muslin cloth. The probe of the pH meter (Five easy plus, METTLER Toledo, USA) was directly immersed in the 100 mL tomato samples to observe the pH of samples in different groups at each sampling time.

S 2.5. Total soluble solids

The total soluble solids (TSS) of filtered tomato juice in each treatment were determined using a digital refractometer (J257 Automatic Refractometer, Rudolph Research Analytical, USA). The refractometer was calibrated with distilled water before analyzing the TSS of samples.

S 2.6. Titratable acidity

For analyzing titrable acidity (TA), 10 mg of filtered tomato juice was mixed with 95 mg of distilled water and a few drops of phenolphthalein indicator. The TA content of tomato samples was determined by titration against NaOH (0.1 N). The acid content of samples was calculated based on the volume of NaOH (0.1 N) used for neutralizing the acid content in tomato samples multiplied with the correction factor of 0.064. The final TA vales were expressed as percent (%) grams of citric acid equivalent per 100 g of a sample according to the following equation.

$$TA = mL\ (NaOH) \times meq_{citric\ acid} \times N\ (NaOH) / W \times 100$$

Where *mL (NaOH)* indicates the volume of NaOH used in titration, $meq_{citric\ acid}$ represents the equivalent value for citric acid (0.0064), *N (NaOH)* is the normality of NaOH, and *W* presents the weight of the sample.

Supplemental Table 1 Optimization of anthocyanin extraction from black plum, blueberry, and wheat bran.

Samples	Extraction method	Extraction parameters*	Total anthocyanin content (mg/Kg)
Black plum (wet basis)	UAE	1000 mL, 30 min	29.70±0.26 ^{a}
		1000+1000 mL, 30+30 min	42.67±0.53 ^{b}
		1000+1000 mL, 45+45 min	40.14±0.35 ^{c}
		1000+1000 mL, 1+1 h	40.14±0.35 ^{c}
	Conventional shaking	1000+1000 mL, 6+5 h	33.22±0.69 d
Blueberry (wet basis)	UAE	1000 mL, 30 min	119.05±0.44 ^{a}
		1000+1000 mL, 30+30 min	194.45±0.35 ^{b}
		1000+1000 mL, 45+45 min	193.19±0.40 ^{b}
		1000+1000 mL, 1+1 h	176.46±0.00 ^{c}
	Conventional shaking	1000+1000 mL, 6+5 h	56.40±0.35 ^{c}
Wheat bran (dry basis)	Conventional shaking	1000+1000 mL, 30 min+5 h	180.68±1.71 ^{a}
		1000+1000 mL, 1.5+5 h	190.49±1.18 ^{b}
		1000+1000 mL, 3+5 h	187.58±1.85 ^{b}
		1000+1000 mL, 6+5 h	210.11±0.65 ^{c}
		1000+1000 mL, 18 + 5 h	209.23±1.12 ^{c}
	UAE	1000 mL, 30+30 min	49.56±0.53 ^{d}

Data were expressed as mean ± standard deviation (n = 3). Means values within the same sample with different superscripts ($^{a-d}$) present statistically significant differences ($p < 0.05$). UAE; Ultrasonic-assisted extraction, * (solvent volume and extraction time)

Supplemental Table 2 Optimization of anthocyanin quantity required to synthesize anthocyanin-loaded lignin nanoparticles (A-LNPs).

Sample	Lignin (mg)	Anthocyanin (mg)	Size (nm)	PDI	Zeta potential (mv)
BP@ALNPs	10	1	133.53±1.14 [a]	0.140±0.004 [a]	-34.10±0.51 [a]
		3	135.80±1.49 [b]	0.157±0.008 [b]	-36.50±1.01 [b]
		5	152.47±1.69 [c]	0.141±0.02 [ac]	-28.70±1.42 [c]
		10	154.40±1.48 [cd]	0.157±0.01 [a]	-34.30±1.73 [ad]
WB@ALNPs		1	126.13±0.94 [a]	0.172±0.01 [a]	-36.27±0.39 [a]
		3	132.40±2.28 [ab]	0.230±0.01 [b]	-36.07±0.58 [ab]
		5	151.07±3.43 [c]	0.130±0.007 [ab]	-34.23±1.07 [ab]
		10	154.97±1.51 [d]	0.210±0.01 [bc]	-33.23±2.37 [c]
BB@ALNPs		1	145.17±1.46 [a]	0.155±0.003 [a]	-36.10±1.10 [a]
		3	152.77±2.21 [b]	0.153±0.019 [b]	-27.30±1.88 [b]
		5	181.63±1.48 [c]	0.399±0.016 [c]	-29.13±0.49 [c]
		10	234.70±3.96 [d]	0.402±0.019 [cd]	-31.93±1.61 [d]

Data were expressed as mean ± standard deviation (n = 3). Means values within the same column with different superscripts ([a-d]) present statistically significant differences ($p < 0.05$) among different quantity of anthocyanin in same sample.

Supplemental Table 3 Encapsulation efficiency of black plum, wheat bran, and blueberry-based A-LNPs.

S.No.	Sample	EE (%)
1	BP@ALNPs	83.18±0.14 [a]
2	WB@ALNPs	72.26±0.15 [b]
3	BB@ALNPs	92.32±1.92 [c]

Data were expressed as mean ± standard deviation (n = 3). Means values within the same column with different superscripts ([a-c]) present statistically significant differences ($p < 0.05$).

Supplemental Table 4 Antioxidant activity of pure anthocyanins and A-LNPs.

Samples		ABTS assay	DPPH assay
		Inhibition (%)	
Purified anthocyanins	Black plum	61.61±0.73 [a]	69.43±0.41 [a]
	Blueberry	60.98±0.94 [ab]	71.00±0.35 [b]
	Bran	62.30±0.98 [ca]	72.24±0.64 [bc]
LNPs	Bare	64.03±0.42 [d]	67.68±0.21 [d]
	BP@ALNPs	80.32±1.07 [f]	85.81±0.69 [e]
	BB@ALNPs	73.32±0.12 [g]	79.56±0.24 [f]
	WB@ALNPs	83.16±1.50 [h]	88.29±0.78 [g]

Data were expressed as mean ± standard deviation (n = 3). Means values within the same column with different superscripts ([a-h]) present statistically significant differences ($p < 0.05$).

Supplemental Table 5 IC_{50} values of bare LNPs, purified anthocyanins, and A-LNPs against *S. aureus*.

S.No.	Samples	*S. aureus* IC_{50} (µg/mL)
1	LNPs	60.22 ± 0.95^{a}
2	WB	32.22 ± 2.09^{b}
3	BP	26.81 ± 1.86^{c}
4	BB	21.18 ± 0.98^{d}
5	WB@ALNPs	17.56 ± 3.85^{e}
6	BP@ALNPs	16.78 ± 2.67^{e}
7	BB@ALNPs	15.82 ± 1.02^{ef}

Data were expressed as mean ± standard deviation (n = 3). Means values within the same column with different superscripts ($^{a-f}$) present statistically significant differences ($p < 0.05$).

Supplemental Table 6 Tensile strength, Elongation at break (%), and WVTR (g m^{-2} h^{-1}) of bare PVA-PEG and PVA-PEG_BB@ALNPs packaging films.

S.No.	Samples	Tensile strength (MPa)	Elongation at break (%)	WVTR (g m^{-2} h^{-1})
1	Bare PVA-PEG film	1.50 ^{a}	13.60 ^{a}	3.85 ± 0.34 ^{a}
2	PVA-PEG_1% BB@ALNPs	1.98 ^{b}	20.36 ^{b}	3.67 ± 0.23 ^{b}
3	PVA-PEG_3% BB@ALNPs	2.56 ^{c}	25.89 ^{c}	2.97 ± 0.16 ^{c}
4	PVA-PEG_5% BB@ALNPs	3.01 ^{d}	28.78 ^{d}	2.89 ± 0.18 ^{cd}
5	PVA-PEG_7% BB@ALNPs	3.52 ^{e}	31.85 ^{e}	2.67 ± 0.12 ^{e}
6	PVA-PEG_10% BB@ALNPs	4.78 ^{f}	35.87 ^{f}	2.45 ± 0.10 ^{f}
7	PVA-PEG_15% BB@ALNPs	8.79 ^{g}	47.52 ^{g}	2.34 ± 0.02 ^{fg}
8	PVA-PEG_20% BB@ALNPs	6.12 ^{f}	38.35 ^{h}	2.32 ± 0.32 ^{fg}

Data were expressed as mean ± standard deviation (n = 3). Means values within the same column with different superscripts ($^{a-g}$) present statistically significant differences ($p < 0.05$).

Supplemental Table 7 Migration test of packaging films

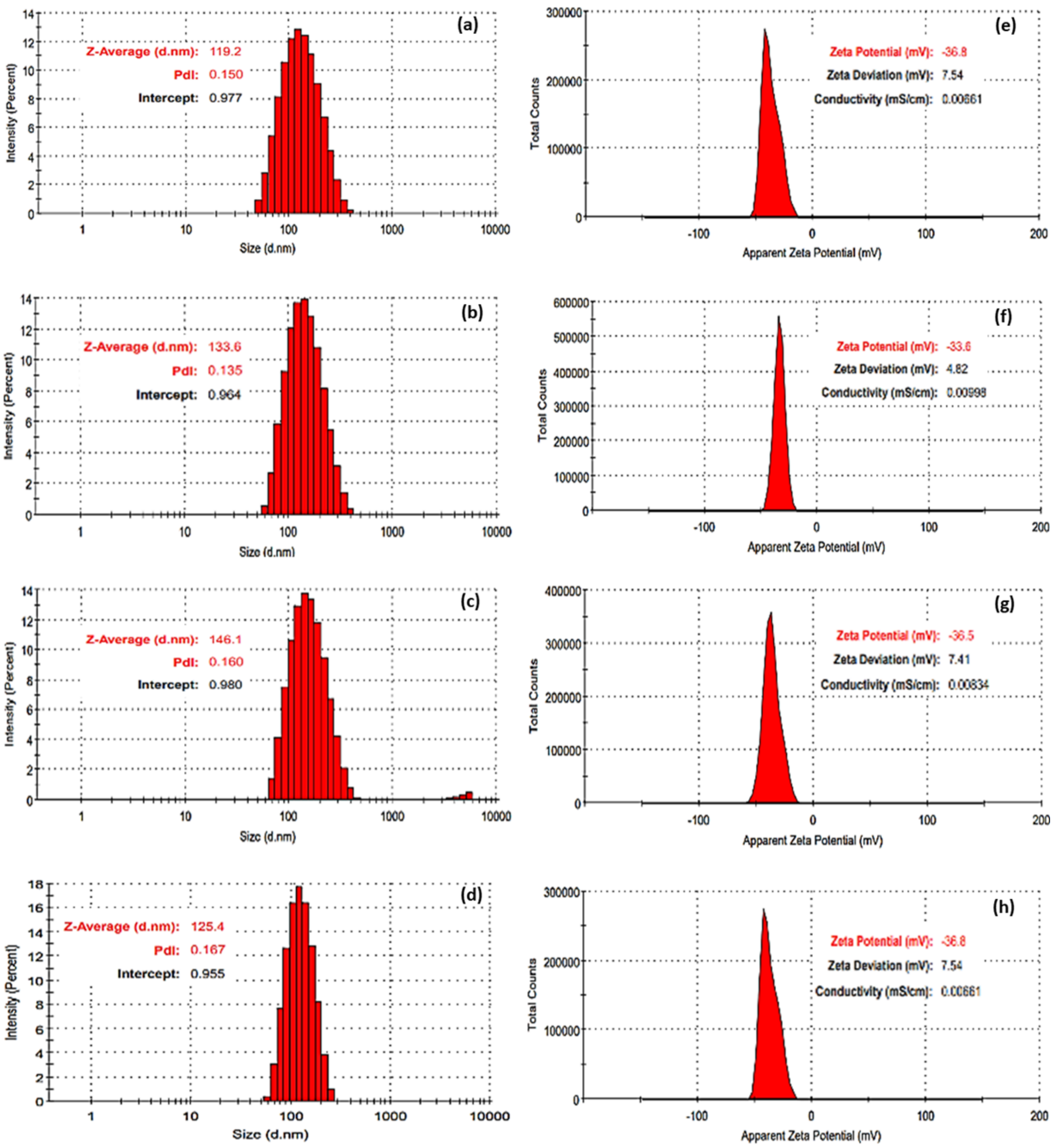
Simulant	PVA-PEG-based film (mg Kg^{-1})	PVA-PEG-15%BB@ALNPs-based film (mg Kg^{-1})
Water	0.57±0.98^{a}	0.37±0.01^{a}
3% acetic acid	0.89±6.50^{a}	0.78±3.70^{b}
50% ethanol	0.12±0.31^{ab}	0.18±0.17^{b}
n-heptane	0.98±0.67^{a}	1.80±0.02^{c}

Data were expressed as mean ± standard deviation (n = 3). Means values within the same column with different superscripts ($^{a-c}$) present statistically significant differences ($p < 0.05$).

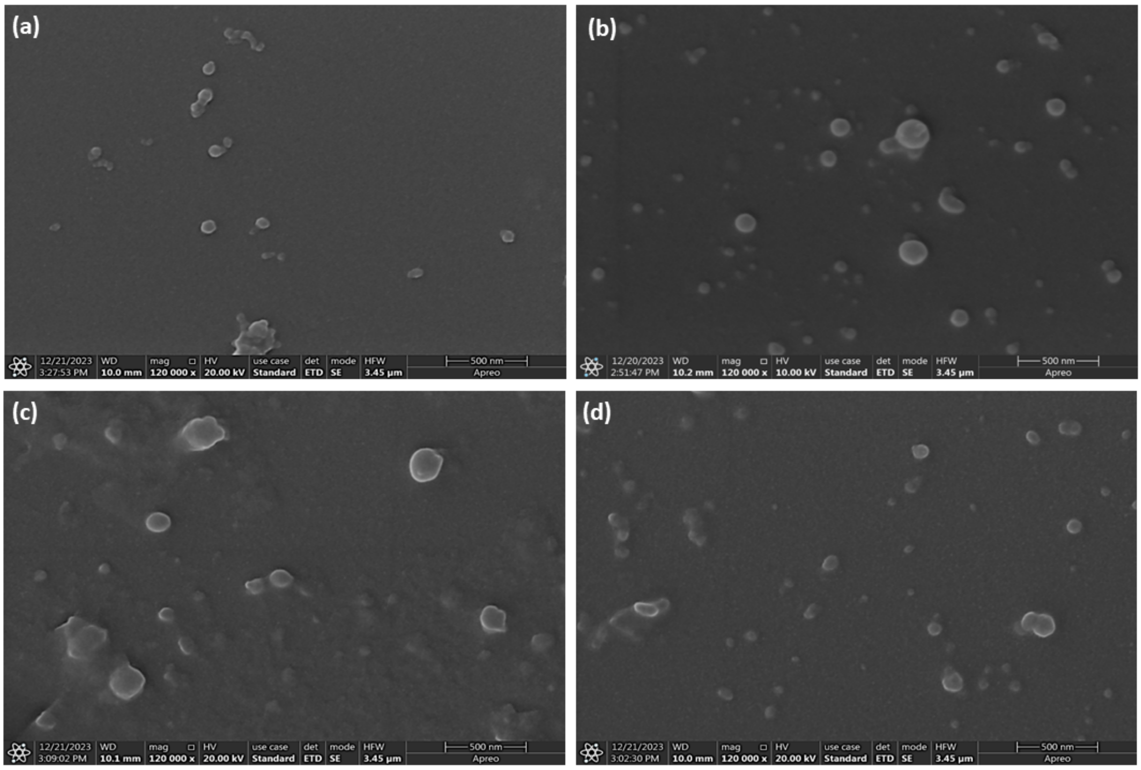
Supplemental Table 8 Postharvest quality analysis of tomato packed with PVA-PEG and PVA-PEG_15%BB@ALNPs film during 15 days of storage.

Sample	Storage days	Weight loss (%)	Firmness (N)	L*	a*	b*	pH	TA (% CA)	TSS (%)
Control	0	0.00±0.00^{Aa}	34.79±2.55^{Aa}	39.10±2.20^{Aa}	29.17±2.09^{Aa}	23.01±2.65^{Aa}	3.77±0.40^{Aa}	0.48±0.02^{Aa}	4.39±0.17^{Aa}
	3	2.51±0.93^{Bb}	30.29±2.80^{Bb}	37.74±2.06^{Bb}	30.31±2.13^{ABb}	22.66±2.64^{ABa}	3.98±0.42^{Aa}	0.41±0.06^{Aa}	4.45±0.16^{Aa}
	6	4.10±0.66^{Cc}	25.84±2.16^{Cc}	36.60±2.62^{Bb}	31.41±1.54^{ABc}	21.29±2.62^{Ba}	4.13±0.10^{Bab}	0.38±0.03^{Bab}	4.56±0.17^{Aab}
	9	7.54±0.55^{Dd}	20.47±3.14^{Dd}	32.87±2.92^{Cc}	32.36±1.39^{Cbc}	18.43±2.19^{Cb}	4.38±0.10^{Bab}	0.34±0.04^{Bab}	4.85±0.19^{Bb}
	12	13.63±0.61^{Ee}	16.98±3.19^{Ee}	30.61±2.06^{Dd}	33.82±1.82^{Cc}	16.23±1.62^{Dc}	4.92±0.13^{Bb}	0.21±0.06^{Cc}	5.42±0.17^{ACc}
	15	21.39±0.75^{Ff}	7.85±3.72^{Ff}	28.60±1.36^{Ee}	35.59±1.78^{Dd}	14.36±2.12^{Ed}	5.20±0.13^{Cc}	0.15±0.05^{Dd}	5.94±0.37^{Ccd}
PVA-PEG film	0	0.00±0.00^{Aa}	34.79±2.55^{Aa}	39.06±2.06^{Aa}	29.61±1.16^{Aa}	23.61±2.61^{Aa}	3.77±0.40^{Aa}	0.48±0.02^{Aa}	4.39±0.17^{Aa}
	3	1.75±0.45^{Bgb}	32.87±1.89^{Bb}	38.02±2.14^{Aa}	30.75±1.19^{ABb}	23.95±2.66^{Aa}	3.94±0.54^{Aa}	0.44±0.02^{Aa}	4.43±0.09^{Aa}
	6	2.74±0.39^{Cb}	28.72±2.28^{Cg}	37.44±1.91^{Bb}	31.25±1.13^{Cbc}	22.49±2.63^{ABa}	4.03±0.55^{ABab}	0.39±0.04^{Aab}	4.48±0.09^{Aa}
	9	4.43±0.50^{Dc}	25.28±3.01^{Dc}	35.21±2.20^{Cb}	31.86±1.44^{Cbc}	21.24±1.54^{Ba}	4.10±0.56^{ABab}	0.35±0.04^{ABab}	4.56±0.08^{Aab}
	12	10.04±0.77^{Eeh}	21.85±3.04^{Ed}	33.16±2.17^{Dc}	32.90±1.83^{CDbc}	20.19±2.48^{Bad}	4.31±0.35^{Bab}	0.31±0.04^{Aab}	4.83±0.11^{Ab}
	15	16.12±1.00^{Feg}	17.53±3.41^{Fe}	31.04±2.37^{Ed}	34.08±2.19^{Ecd}	18.60±2.30^{Cb}	4.72±0.31^{Bb}	0.27±0.06^{Cc}	5.24±0.18^{Bc}
PVA-PEG_BB@ALNPs	0	0.00±0.00^{Aa}	34.79±2.55^{Aa}	39.92±1.62^{Aa}	29.01±1.55^{Aa}	23.12±1.29^{Aa}	3.77±0.40^{Aa}	0.48±0.02^{Aa}	4.39±0.17^{Aa}
	3	1.00±0.20^{Bi}	33.27±1.79^{Aa}	38.88±1.80^{Aab}	29.92±1.27^{Aa}	23.45±1.27^{Aa}	3.81±0.05^{Aa}	0.45±0.01^{Aa}	4.32±0.14^{Aa}
	6	1.56±0.31^{Bgb}	31.28±1.61^{Bb}	37.92±1.36^{Bb}	30.57±1.06^{ABb}	22.70±1.25^{ABa}	3.85±0.06^{Aa}	0.44±0.02^{Aa}	4.39±0.12^{Aa}
	9	3.29±0.58^{Cbj}	29.60±2.01^{Cg}	37.06±1.88^{Bb}	30.87±1.52^{ABb}	22.69±1.29^{ABa}	3.86±0.20^{Aa}	0.42±0.02^{Aa}	4.45±0.14^{Aa}
	12	4.53±0.51^{Dc}	27.52±2.31^{Dg}	35.95±1.29^{Cb}	31.54±1.55^{Bbc}	21.96±1.52^{Ba}	4.08±0.57^{Bab}	0.39±0.02^{ABa}	4.69±0.19^{Aab}
	15	6.54±1.11^{Ek}	24.76±2.19^{Ec}	34.58±1.11^{Cb}	31.99±1.35^{Bbc}	21.07±1.67^{Ba}	4.15±0.57^{Bab}	0.36±0.03^{ABab}	4.71±0.18^{Ab}

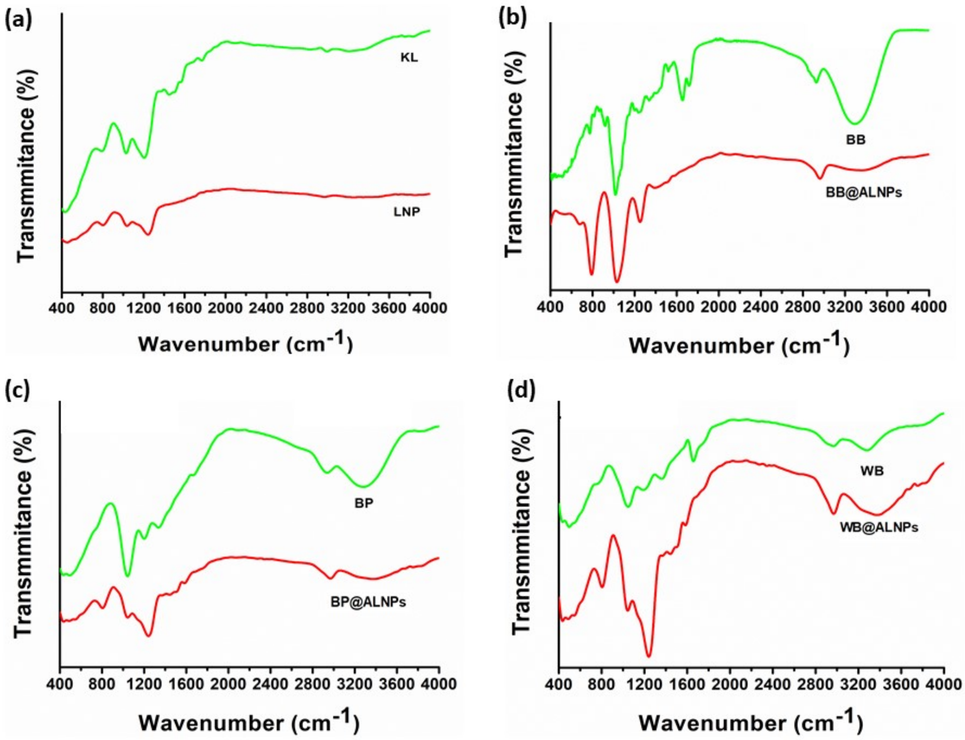
The results were expressed as mean ± standard deviation (n=10), and letters ($^{a-k}$) indicated significant differences ($p < 0.05$) between control, samples packaged with PVA-PEG and PVA-PEG_BB@ALNP film, and letters ($^{A-F}$) indicated significant differences between samples at different storage days ($p < 0.05$).



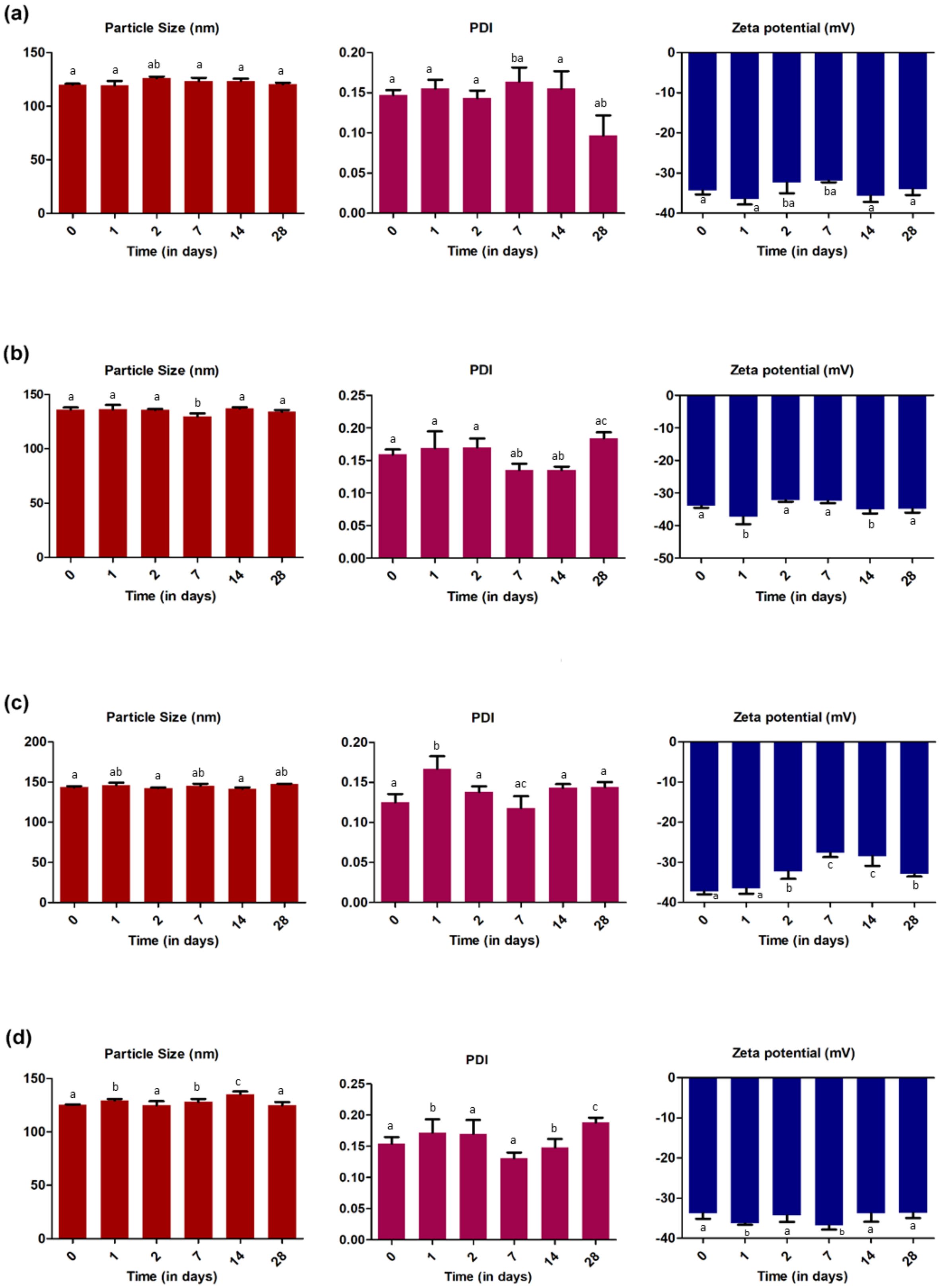
Supplemental Fig. 1 Particle size (a) bare LNPs, (b) BP@ALNPs, (c) BB@ALNPs, (d) WB@ALNPs, and Zeta potential of (e) bare LNPs, (f) BP@ALNPs, (g) BB@ALNPs, (h) WB@ALNPs.



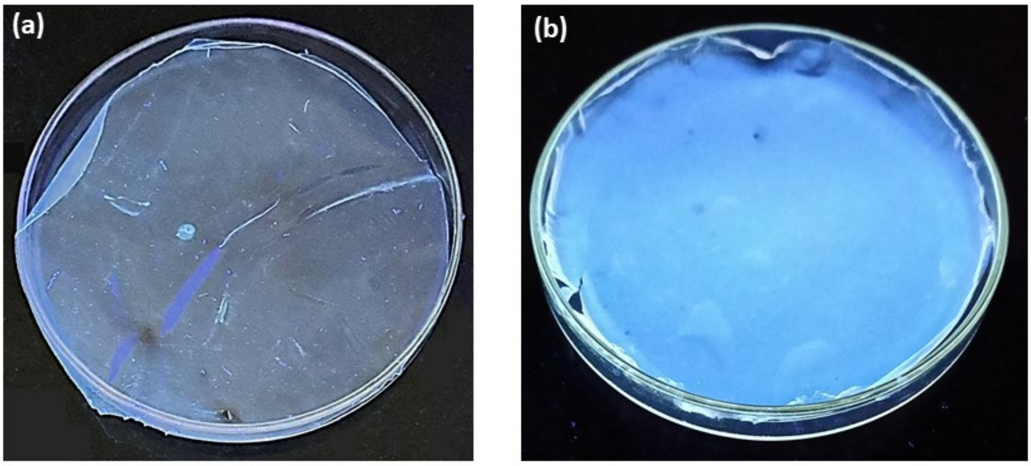
Supplemental Fig 2 SEM analysis of (a) bare LNPs, (b) BP@ALNPs, (c) BB@ALNPs, and (d) WB@ALNPs.



Supplemental Fig. 3 FTIR spectrum of (a) kraft lignin (KL) and LNPs, (b) purified BB anthocyanin and BB@ALNPs, (c) purified BP anthocyanin and BP@ALNPs, and (d) purified WB anthocyanin and WB@ALNPs.



Supplemental Fig. 4 Stability study of (a) bare LNPs, (b) BP@ALNPs, (c) BB@ALNPs, and (d) WB@ALNPs.



Supplemental Fig. 5 Fluorescence behavior of (a) PVA-PEG-based film, and (b) PVA-PEG-15%BB@ALNPs-based film exposed to UV light at 365 nm.