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Research article

Antimicrobial screening of pecan shell extract and efficacy of pecan shell extract-pullulan coating against *Listeria monocytogenes, Salmonella enterica,* and *Staphylococcus aureus* on blueberries

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ABSTRACT

Pecan shell is considered an agricultural waste; however, it contains various bioactive compounds with potential inhibitory effect against microorganisms. This study evaluated the antimicrobial efficacy of pecan shell extract (PSE) *in vitro* using disc-diffusion method and *in vivo* on blueberries as an antimicrobial coating using pullulan. For *in vitro* study, 5 and 10 % of aqueous (A-PSE) or ethanol pecan shell extract (E-PSE) incorporated into pullulan film were tested against different bacterial and fungal strains. Pullulan film disc was used as control. The diameter of growth inhibition (mm) around discs was measured. For *in vivo* study, PSE-P (5 % w/v aqueous pecan shell extract+5 % w/v pullulan), P (5 % w/v pullulan) or control (water) were spray coated on blueberries inoculated (~5 log CFU/g) with *Listeria monocytogenes, Salmonella enterica or Staphylococcus aureus*; and stored at 4 °C, 50 ± 10 % RH for 15 days. The effect of antimicrobial coating against pathogens and its impact on quality during storage were determined. A-PSE and E-PSE films were more effective against Gram-positive bacteria and showed no antifungal effect at tested concentrations when evaluated *in vitro*. Immediately after coating on blueberries, PSE-P significantly reduced *Listeria monocytogenes* by 2 log CFU/g and lowered survival until day 5 than control or P. Native aerobic microbial population was reduced ($P < 0.05$) by 0.7 log CFU/g immediately after coating. PSE-P coating or storage time had no significant effect ($P > 0.05$) on the survival of *S. enterica, S. aureus* and native fungal population. PSE-P maintained the firmness of blueberry with no significant effect on its color, TSS, and pH during 15 days storage. PSE-P significantly reduced ($P < 0.05$) the spoilage rate by 21 % than control (28.5 %) and P (37 %); and minimized ($P < 0.05$) weight loss during storage. Pecan shell extracts show promise as a potential antimicrobial compound whose application on various food products or packaging material could be further explored.

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1. Introduction

United States is one of the major pecan producers in the world. In 2023, annual production totaled 271 million pounds [1] and it is likely increasing over the years as the demand is growing. The shells make up a major portion of the nut, almost 40 %–60 %, depending on cultivar [2] which amounted to almost 163 million pounds produced as waste/by-product from pecan industry in 2023 alone, and has very little use or economic importance.

The pecan shells, however, has potential to be a valuable by-product due to the presence of high amounts of phenolic and flavonoid compounds exhibiting antioxidant and antimicrobial property [3]. Past studies have shown that shells contain 5-8 times more phenolics and flavonoids and 10–20 times more proanthocyanidins than the pecan kernel [4,5]. The major components in pecan shell extracts responsible for its antioxidative and antimicrobial properties are phenolic acids like gallic, caffeic, vanillic, ellagic, and, p-hydroxy benzoic acid; flavonoids like epicatechin, epigallocatechin, and, epigallocatechin gallate [4–7] and lignin degradation products like lignols, dilignols, trilignols, and oligolignols [8].

Growing concern among consumers regarding use of chemical preservatives in food has raised awareness towards including natural additives instead. In addition to preserving the food, natural preservatives like phenolic compounds also exhibit health beneficial property [9] thus serving a dual-function effect. Several studies have explored the potential of using pecan shell extracts as a natural antimicrobial by direct applications of dry or liquid extract in foods like lettuce [10], catfish filets, cantaloupes [11], chicken skin [12] and also evaluated its potential for quality improvement of beef patties [3]. However, there are limited literature on application of pecan shell extracts in the form of antimicrobial coating for fresh produce against several food borne pathogens and evaluating its effect on shelf-life.

Pullulan is a polysaccharide produced by fungi, *Aureobasidium pullulans,* that has been extensively studied for its potential use as a natural coating for food and food packaging [13]. It has been regarded as Generally Recognized as Safe (GRAS) substance by FDA for nearly 2 decades [14]. Some of the characteristic features of pullulan as a coating material are: it is colorless, odorless, tasteless, non-toxic, has good gas barrier property and has high film forming ability [13]. Pullulan coating have shown beneficial effect on shelf-life extension and quality of various food products [13,15]. However, it does not have antimicrobial property of its own thus, incorporation of antimicrobial compounds can give an added benefit of reducing the risk of pathogen growth on the coated surface [16–18].

Fresh and frozen blueberries have been associated in numerous outbreaks of foodborne disease majorly due to virus and parasite like Norovirus, Hepatitis A and *Cyclospora cayetanensis;* however, they are susceptible to bacterial pathogen contamination at any point of production. In the past, blueberries have been associated with outbreaks due to *Listeria monocytogenes* [19], *Salmonella* Muenchen and Newport [20], and, *Escherichia coli* O26 [21] resulting in several illness. Due to the perishable nature of berries, they are usually directly packed in the retail container while in the field or packed in packinghouse without undergoing any washing steps [22]. *Staphylococcus aureus* is one of the major commensal organisms found on the skin of humans and because of the of hand-picking and handling nature of blueberries, food handlers can be a vector for transferring this organism to its surface as well. Furthermore, due to high moisture content in blueberry and delicate skin it is prone to spoilage and bacterial contamination. Thus, appropriate intervention strategies to mitigate these risks on minimally processed foods like blueberries is important.

To our knowledge, this is the first paper to test antimicrobial efficacy of aqueous pecan shell extracts-pullulan coating for safety and quality of blueberries. The major objectives of this study were i) to evaluate antimicrobial activity of aqueous and ethanolic extracts of pecan shell against several bacterial pathogens and mold, ii) to determine efficacy of aqueous pecan shell extract-pullulan antimicrobial coating against food-borne pathogens like, *S. aureus, S. enterica* and *L. monocytogenes* on blueberries during 15 days refrigerated storage and; iii) to evaluate the coating's impact on quality of blueberry (color, texture, pH, TSS, spoilage rate and weight loss) and on its native microbial population during the refrigerated storage.

2. Materials and methods

2.1. Preparation of pecan shell extract (PSE)

Pecans (*Carya illinoinensis*) of "Caddo" variety were provided by Louisiana Pecan Growers Association during the October/November season of 2019/2020 and stored at 4 °C until use. Higher level of phenolics and antiradical activity detected in "Caddo" among other pecan varieties grown in Louisiana [8] was the basis for its selection. Preparation of PSE was carried out as per the protocol by Yemmireddy et al. (2020) [11]. Briefly, pecan shell powder was defatted using hexane 1:20 (w/v) and constantly mixed at 160 rpm, 23 °C, dark conditions for 45 min on a shaker incubator (Model C25KC, New Brunswick, Edison, NJ, USA). The defatted powder was vacuum filtered using Whatman® No. 1 filter paper, and the process was repeated twice. PSE were then prepared using 2 extraction methods: aqueous and ethanolic. For aqueous extract, defatted shell powder was added to boiling distilled water (1:20 w/v) in hot water bath (Southbend tilting skillet, Model BTT-16G, Fuquay Varina, NC) maintained at 98 ± 3 °C and constantly mixed for 30 min. The mixture was cooled and filtered through Whatman® No. 1 filter paper. For ethanolic extract, defatted pecan shell powder was constantly mixed with ethanol (1:20 w/v) at 160 rpm, 22 °C, dark conditions for 1 h and vacuum filtered through Whatman® No. 1 filter paper. Ethanolic infusion was transferred to round bottom flask and ethanol was evaporated in a rotary evaporator (Brinkman/Buchi RE-111 Rotavapor). Both the extracts were then lyophilized using a Genesis Pilot freeze dryer (VirTis™, SP Scientific, Warminster, PA, USA). The PSE powder was stored in amber colored glass bottles at −20 °C until further use.

2.2. Preparation of pecan shell extract (PSE)-pullulan film

PSE concentration of 5 and 10 % (w/v) was selected as per the minimum inhibitory and minimum bactericidal concentration observed for the extracts from our previous lab study [11]. A film-forming solution (g/100 mL) was prepared by mixing pullulan (10 % w/v), glycerol (5–10 % w/v), and aqueous PSE (A-PSE) or ethanol PSE (E-PSE) each at 5 and 10 % (w/v) of film forming solution in an electromagnetic stirrer (Model ES24, Wigo, Poland) at 45 °C, 500 rpm for 20 min. After mixing, 10 mL of solution was aseptically transferred to sterile petri dishes (dia. 90 mm) and the films were dried at 37 °C for 12 h and kept in humidity chamber at 30 % RH until the experiment. A control i.e., pullulan solution without PSE was also prepared.

2.3. Antimicrobial screening of PSE-pullulan film

Agar disc diffusion method was used to screen antibacterial and antifungal property of PSE-pullulan film according to Gniewosz et al. (2014) [23] with slight modification. The antimicrobial properties were tested against Gram positive bacteria (*Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 7644)), Gram negative bacteria (*Salmonella* Enteritidis (ATCC 13076), *Escherichia coli* O157:H7 (ATCC 700728)), and mold strains *(Penicillium chrysogenum* (ATCC 10136), *Rhizopus stolonifer* (ATCC 14037), *Aspergillus niger* (ATCC 9142), *Fusarium solani* (ATCC 36031)). The cultures were obtained from the pure cultures collection of the Department of Food Biotechnology and Microbiology (Warsaw University of Life Sciences – SGGW, Warsaw, Poland). Bacterial cultures were grown on nutrient agar (NA) (BTL, Lodź, Poland) and then transferred to nutrient broth (NB) (BTL, Lodź, Poland) each at 37 °C for 24 h while mold on Sabouraud agar (SDA) (BTL, Lodź, Poland) at 22 °C until spore formation (about 14 days). Bacterial inocula and mold spore suspensions were prepared in sterile 0.85 % saline solution (NaCl) (POCH, Gliwice, Poland) to achieve 0.5 McFarland ($\sim 10^8$ CFU/mL), using densimeter (Densimat, bioMérieux, Marcy l'Etoile, France) for bacteria and $\sim 10^6$ spores/mL, using hemacytometer for mold spores. The suspensions of bacteria were spread evenly with sterile cotton swabs on Mueller Hinton Agar (MHA) (Merck, Darmstadt, Germany) and mold were spread on SDA. The PSE-pullulan film (A-PSE or E-PSE) and control film were cut to form a disc of 6 mm diameter using a circular knife and were applied on inoculated media surfaces. MHA was incubated at 37 °C for 24 h, while SDA at 28 °C for 72 h. After incubation, the diameter of growth inhibition around the discs was measured and the result was expressed in mm (including 6 mm disc dia.). The experiment was conducted in triplicates.

2.4. Preparation of bacterial strains

For antimicrobial coating study, *Listeria monocytogenes* (101 M serotype 4b, V7 serotype 1/2a, LCDC 81–861 serotype 4b), obtained from Dr. Michelle Danyluk at the University of Florida; *Salmonella enterica* serovar Typhimurium (ATCC 14028), *Salmonella enterica* serovar Montevideo (ATCC 8387), and *Staphylococcus aureus* (ATCC 25923, ATCC 12600) obtained from the American Type Culture Collection (ATCC) were used. All bacterial strains were adapted to grow in 50 μg/mL nalidixic acid [24]. The strains were stored in Tryptic Soy Broth (Criterion™, Hardy Diagnostics, Santa Maria, CA, USA) with 50 μg/mL nalidixic acid (TSBN) (*S. enterica* and *S. aureus*) or TSBYN (TSBN with 0.6 % yeast extract for *L. monocytogenes*) containing glycerol (70:30 v/v, culture:glycerol) at −80 °C for long-term preservation [25] and the frozen cultures were activated in three successive steps. Briefly, 0.1 mL of individual strains were first activated in its respective broth 9 mL of TSBN or TSBYN and incubated for 24 h at 37 °C. Then, a loopful from the overnight grown inoculum was streaked on its respective selective agar media plates and incubated for 24 h at 37 °C. *S. enterica* was grown in Xylose Lysine Deoxycholate (XLDN) (Criterion™, Hardy Diagnostics, Santa Maria, CA, USA), *L. monocytogenes* was grown in Oxford Agar Base (OXN) (Criterion™, Hardy Diagnostics, Santa Maria, CA, USA) and *S. aureus* was grown in Baird-Parker (BPN) agar base supplemented with egg yolk emulsion and 1 % potassium tellurite solution (HiMedia Laboratories, Mumbai, India). Finally, a loopful of pure colony from the selective media was inoculated in TSBN or TSBYN and incubated for 24 h at 37 °C. All media were supplemented with 50 μg/mL nalidixic acid. The cells from overnight grown inoculum were harvested by centrifuging at 4900 rpm for 5 min, the supernatant was decanted, and the pellets were washed with sterile 0.1 % peptone water (Difco™, Becton Dickinson, Sparks, MD, USA). The process was repeated two times. Finally, equal volume of each strain were combined to form an inoculum cocktail, adjusted to a final volume of 120 mL using sterile 0.1 % peptone water, and used as the inoculum (*L. monocytogenes*: 9.09 ± 0.87 log CFU/mL, *S. enterica*: 7.77 ± 0.33 log CFU/mL, and, *S. aureus*: 7.57 ± 0.49 log CFU/mL).

2.5. Sample preparation and inoculation

Blueberries were purchased at a local supermarket, stored at 4 °C and used within 2 days of purchase. Blueberries (3.5 ± 1 g) of uniform size with no physical damage and fungal infection were selected for the study. The berries (1200 ± 1 g) were dip inoculated in 120 mL bacterial suspension (0.1 mL inoculum/g berries) inside sterile 55 oz. sample bag (VWR®, PA, USA) for 3 min with gentle mixing. To retain texture and reduce stress in berries while dip inoculating, berries were divided and placed into 4 sample bags (300 g/bag). The inoculum was discarded after 3 min, and the blueberries were dried for 2 h on a sterile tray inside a biosafety cabinet.

2.6. Coating treatment and storage

The inoculated berries were subjected to different coating treatments: 5 % aqueous PSE +5 % pullulan solution (PSE-P), 5 % pullulan solution (P), or a control treatment using sterile distilled water (control). The selection of the concentration and extraction method for PSE was based on the results obtained from the antimicrobial screening study. Additionally, from our preliminary study, 5

% concentration of pullulan was selected for the coating solution as it ensured appropriate spray coating consistency without making solution too thick (data not shown). Pullulan coating solution (5 g/100 mL) was prepared by dissolving pullulan (Spectrum Chemical Mfg. Corp., New Brunswick, NJ, USA) and glycerol (15 g/100 g pullulan) in sterile distilled water at 45 °C, 500 rpm for 20 min. Glycerol aids as a plasticizer to improve the flexibility of coating. For PSE-P, to the pullulan solution, PSE powder (5 g/100 mL) was added and mixed in sterile condition for further 20 min. The mixtures were homogenized using benchtop homogenizer (Fisherbrand™ 850, Waltham, MA, USA) at 20,000 rpm for 2 min to prepare final coating solution.

Inoculated berries (400 ± 1 g) were placed on sterile bin and spray coated (0.016 mL treatment solution/g berries) using airbrush (Paasche H-100D, Kenosha, WI, USA) with 0.64 mm nozzle from a height of 8 cm. The berries were gently swirled in the bin while coating to facilitate uniform coating on all sides. Berries were also sprayed with sterile distilled water as control. The coated berries were dried inside biosafety cabinet at room temperature for 1 h. After drying, the samples were packaged in polyethylene terephthalate (PET) clamshell box (60 ± 3 g per box) and stored at 4 °C, 50 ± 5 % RH, L = 0 (no light setting) for 15 days in growth chamber (MLR-352H-PA, phcbi, Wood Dale, IL, USA). The sampling was done immediately after coated samples dried on day 0 and on day 2, 5, 8, 11 and 15.

2.7. Bacterial enumeration

Clamshell boxes with blueberries (PSE-P, P and control) were removed from storage on each sampling day and 25 g of berries were placed in a sterile, 24 oz. Whirl-Pak (Nasco, Fort Atkinson, WI, USA) filter stomacher bag and diluted 1:4 (w/v, berries:broth) with Dey-Engley (DE) neutralizing broth (Criterion™, Hardy Diagnostics, Santa Maria, CA, USA). The sample was pummeled in stomacher (BagMixer® 400S, Interscience, Woburn, MA, USA) for 1 min at a speed of 3. The homogenate samples were serially diluted 10-fold with sterile 0.1 % peptone water. Enumeration was done on respective selective agar. *L. monocytogenes* and *S. enterica* were plated on OXN and XLDN, respectively, incubated at 37 °C for 24 h whereas, *S. aureus* on BPN at 37 °C for 48 h. Independent trials were conducted twice starting from purchasing blueberries to enumeration. In each trial, sampling was done twice for all treatments, from same clamshell box, giving a total of 4 measurements (n = 4) per treatment per organism.

2.8. Quality evaluation of pecan shell extract-pullulan coated blueberries

Medium-sized, uninoculated, and bruise-free blueberries were spray coated with PSE-P, P or control and stored at 4 °C, 50 ± 5 % RH, and L = 0 (no light setting) for 15 days in the incubator as explained in section 2.6. Sampling was done on day 0, 2, 5, 8, 11, and 15 and tested for post-harvest quality parameters like color, total soluble solids (TSS), pH, native microbial population, texture, weight loss, and spoilage rate. Two trials with duplicate sampling in each trial was performed.

2.8.1. Color, texture, total soluble solids (TSS) and pH

On each sampling day, six blueberries from one clamshell box were randomly selected and placed on the petridish (CM-A128) and color was measured using hand-held colorimeter (CR-400 Chroma Meter, Konica Minolta Inc., Osaka, Japan) calibrated with standard white tile. The process was repeated three times for each replicate. L*(0 = black and 100 = white), a*(+a* = redness, -a* = greenness), and b*(+b* = yellow, -b* = blue) were measured. Total color difference (ΔE) was calculated using the following equation [26], where control from day 0 was taken as the reference. Results were reported as average ± standard deviation (n = 6).

$$\Delta E^{*} = \sqrt{\left(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}\right)} \tag{1}$$

where, $\Delta L^* = (L_1^* - L_0^*)$; $\Delta a^* = (a_1^* - a_0^*)$; and $\Delta b^* = (b_1^* - b_0^*)$

The texture of coated blueberries and control samples were measured using a texture analyzer (TA.XT PlusC, Stable Micro Systems, Surrey, UK) calibrated with a 5-kg load cell following the protocol described by Zhang, Tsai, and Tikekar (2021) [27]. Briefly, blueberries (n = 8) were sampled from the same clamshell box that was used to measure color. Cylindrical flat probe (TA-25 probe, 2 in. diameter) was lowered at a test speed of 1 mm/s with pre and post-test speed of 5 mm/s each to deform the berry by 3 mm. Results were expressed as average ± standard deviation (n = 16) of maximum peak force (firmness, N) recorded from each measurement.

After the color and texture measurement, blueberries from the same clamshell box were manually macerated in mortar and pestle and used for TSS (%) and pH measurement using digital hand-held refractometer (VWR® International, Radnor, PA, USA) and hand-held pH meter (Model HI 98121, Hanna instruments, Woonsocket, RI, USA), respectively.

2.8.2. Native microbial population

Blueberries (10 g) were placed in sterile, 24 oz. Whirl-Pak (Nasco, Fort Atkinson, WI, USA) filter stomacher bag and diluted with 90 mL sterile 0.1 % peptone water. The sample was pummeled in stomacher (BagMixer® 400S, Interscience, Woburn, MA, USA) for 1 min at a speed of 3. The homogenate samples were serially diluted 10-fold with sterile 0.1 % peptone water. For aerobic bacterial count, 3 M Petrifilm™ Aerobic Count Plates (3 M Health Care, MN, USA) were used and incubated at 35 °C for 48 h and for determining Yeast and Mold counts, 3 M Petrifilm™ Yeast and Mold Count Plates (3 M Health Care, MN, USA) were used and incubated at 28 °C for 3–5 days. The results were expressed in log CFU/g.

2.8.3. Weight loss and spoilage rate

For each treatment, two clamshell boxes each containing 50 blueberries were weighed at the start of the experiment (day 0) and

Table 1
Antibacterial and antifungal activity of aqueous pecan shell extract (A-PSE) and ethanol pecan shell extract (E-PSE) in pullulan film.

PSE	Extract concentration in film-forming solution (%)	Bacterial strains				Mold strains			
		E. coli O157: H7 ATCC 700728	*Salmonella* Enteritidis ATCC 13076	*Staphylococcus aureus* ATCC 25923	*Listeria monocytogenes* ATCC 7644	*Penicillium chrysogenum* ATCC 10136	*Rhizopus stolonifer* ATCC 14037	*Aspergillus niger* ATCC 9142	*Fusarium solani* ATCC 36031
	[a]inhibition zone (mm) ± SD								
Aqueous extracts (A-PSE)	0	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
	5	**6.00 ± 0.00 b**	10.15 ± 0.79 b	15.18 ± 0.69 c	15.67 ± 1.26 b	N/D	N/D	N/D	N/D
	10	8.51 ± 0.41 a	10.40 ± 0.29 ab	15.89 ± 0.20 bc	18.13 ± 0.68 a	N/D	N/D	N/D	N/D
Ethanol extracts (E-PSE)	0	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
	5	**6.00 ± 0.00 b**	9.6 ± 0.80 b	16.71 ± 1.16 b	18.45 ± 1.31 a	N/D	N/D	N/D	N/D
	10	8.72 ± 1.37 a	11.13 ± 1.35 a	19.62 ± 2.90 a	18.09 ± 0.75 a	N/D	N/D	N/D	N/D

Mean values ± SD with different letters within same column are significantly different ($P < 0.05$).
Inhibition zone with bold letters represent that organisms were only inhibited under the film and no inhibitions observed around the disc.
N/D represents no inhibition zone was observed around the disc.
[a] The zone of inhibition included the disk diameter of 6.0 mm.

then on each sampling day throughout the storage period. The experiment was done in duplicate. Weight loss (%) was expressed as percent loss of initial total weight. The clamshell boxes were also visually observed for mold decay, shrinkage, and any visible decay to evaluate overall spoilage condition. Spoilage rate (%) was calculated by dividing the total number of spoiled berries by total number of blueberries in the box. Pictures were also taken to record the appearance of blueberries.

2.9. Statistical analysis

All the data were analyzed using linear mixed model (Proc mixed feature) in SAS version 9.4 (SAS Institute Inc., Cary, NC). For the antimicrobial coating study, the fixed effects were time, treatment and interaction term time*treatment, the random effects were replicates and replicates*treatment and to account for the repeated measures an autoregressive heterogeneous ARH (1) residual structure was used. For all data, the Fisher's least significant difference test was used to determine the significant differences in mean value with significance considered at $P < 0.05$.

3. Results and discussion

3.1. Antimicrobial screening of pecan shell extracts

Efficacy of pecan shell extract in pullulan film against several Gram-positive, Gram-negative bacteria and molds is presented in Table 1. The aim of this *in vitro* test was also to select an appropriate extraction method and concentration of the pecan shell extract for incorporation into pullulan for the *in vivo* antimicrobial coating study. Aqueous and ethanol extracted pecan shell extracts showed antibacterial activity at both 5 and 10 %; however, no antifungal activity was observed at the tested concentrations. Control i.e., pullulan film by itself did not exhibit growth inhibition. Among the tested bacterial strains, zone of inhibition was higher for the Gram-positive bacteria (*S. aureus,* 15.18–19.62 mm and *L. monocytogenes,* 15.67–18.45 mm) than the Gram-negative bacteria (*S. enterica,* 9.6–11.13 mm and *E. coli* O157:H7, 6–8.72 mm). Similar trends were reported in the past where, 1 % (10 g/L) aqueous pecan shell extract effectively inhibited Gram-positive food-borne pathogenic bacteria (*L. monocytogenes, L. innocua, S. aureus,* and *Bacillus cereus*), but had little effect against Gram-negative bacteria (*Salmonella* Enteritidis*, Pseudomonas aeruginosa, Aeromonas hydrophila*), and no impact on *E. coli* and tested fungi [10]. Likewise, another study reported that both aqueous and ethanolic pecan shell extracts (0.15–2.5 mg/mL) inhibited the growth of *L. monocytogenes, S. aureus, Vibrio parahaemolyticus,* and *Bacillus cereus*, but showed no effect on *E. coli* and fungal strains for either extract [7]. The previous work from our lab on evaluating minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for the pecan shell extracts also showed that *E. coli* O157:H7 was the most resistant organism with $\geq$ 5 mg/mL MIC and MBC values followed by *S. enterica* and *L. monocytogenes* (1.25–5 mg/mL) [11] which are analogous to the trend observed in the present disc-diffusion study. Although MIC for aqueous pecan shell extracts were $\geq$5 mg/mL, no inhibitory effect for *E. coli* at 5 % in disc-diffusion study could be due to decrease in concentration as we move further from the antimicrobial disc compared to the micro dilution method done for MIC studies where exact dilutions provide concise concentration [5].

The antioxidative and antimicrobial properties of shell extracts come from phenolic acids like gallic acid, caffeic, vanillic acid and ellagic acid and lignin degradation products like lignols, dilignols, trilignols, and oligolignols [4,5,8]. These phenolic compounds are hydrophobic in nature. Studies have demonstrated that Gram-positive bacteria are more susceptible to plant extracts compared to Gram-negative bacteria, primarily due to the absence of an outer membrane surrounding the lipopolysaccharide wall, which otherwise hinders the penetration of these hydrophobic phenolic compounds [28]. Additionally, phenolic compounds can cause a reduction in the internal cell pH, leading to damage to the bacterial cell membrane [29]. The resistance displayed by *E. coli* and tested fungal strains in this study could also be attributed to their ability to utilize tannins and their monomers and dimers present in pecan shells [7] as a carbon source, given their capacity to grow in the presence of these compounds [30]. Increasing the extract concentration from 5 to 10 % showed significant ($P < 0.05$) inhibition against *L. monocytogenes* and *E. coli* by aqueous extracts, while ethanol extracts significantly inhibited *S. aureus* and *E. coli*. Overall, ethanolic extracts exhibited greater ($P < 0.05$) inhibition which could be due to its higher total phenolic content than aqueous extracts [8]. In contrast, other studies have reported higher phenolic content and antioxidant activity for aqueous extracts than ethanolic extracts of pecan shells [7] which could be due to varietal and extraction process difference.

Based on the *in vitro* results, aqueous pecan shell extracts were chosen to incorporate into pullulan, evaluating their potential as an antimicrobial coating for blueberries. Water-based extraction was selected based on several advantages. For example, water as an extraction solvent is non-toxic, environmentally friendly, and cost effective thus its use has been increasing rapidly in the past decade [31]. Furthermore, aqueous extraction also results in significantly higher yield of extract than ethanolic extraction [29,32]. The study by Cason et al. (2021) reported an average of 250 mg dry extract/g defatted shell powder using hot water extraction, compared to only 166 mg dry extract/g defatted shell powder with ethanolic extraction [8]. Additionally, previous research found no significant difference between aqueous and ethanol extracts in reducing pathogens like *L. monocytogenes* on food matrices like catfish filets and cantaloupe [11]. In our preliminary experiments (data not shown), a 5 % aqueous extract concentration with pullulan successfully formed a coating solution without excessive viscosity; therefore, 5 % aqueous extract was selected.

Our findings showed that both aqueous and ethanolic extracts possess antimicrobial property, especially against *L. monocytogenes, S. aureus, S. enterica* and least against *E. coli* and the inhibition is proportional to extract concentration. Application of 5 % aqueous pecan shell extract with pullulan as an antimicrobial coating on blueberries and its effect on the quality was further studied.

3.2. Effect of pecan shell extract-pullulan coating against pathogens on blueberries

Immediately after inoculation and drying (day 0), levels of pathogens on blueberries were 5.94 ± 0.07 log CFU/g (*L. monocytogenes),* 5.05 ± 0.26 log CFU/g (*S. enterica*) and 5.56 ± 0.48 log CFU/g (*S. aureus*) (Fig. 1). After blueberries were spray coated with control, P or PSE-P, on day 0, *L. monocytogenes* declined by 0.53–2.06 log CFU/g, *S. enterica* by 1.06–1.3 log CFU/g, and, *S. aureus* by only 0.6–0.79 log CFU/g. Compared to water wash (control) and P coating on day 0, PSE-P significantly reduced ($P < 0.05$) *L. monocytogenes* by 2.06 log; however, no significant effect was observed against *S. enterica* (1.09 log CFU/g) and *S. aureus* (0.6 log CFU/g). During refrigerated storage, survival of *L. monocytogenes* in control and P coated blueberries were not significantly different; however, PSE-P significantly reduced ($P < 0.05$) the survival of *L. monocytogenes* until day 8. Gallic acid is one of the main phenolic components in water extracted pecan shell extract [8], and due to its electrophilic nature, it has demonstrated potential to cause permanent change in bacterial membrane properties, and cause local rupture or pore formation in cell membranes leading to leak of essential intracellular constituents for bacteria like *E. coli, L. monocytogenes, Pseudomonas aeruginosa* and *S. aureus* [33]. Pecan shell extracts (aqueous and ethanol extracted) when directly applied on food surfaces have shown to reduce *L. monocytogenes* by 0.2–2.8 log CFU/g on catfish filets and cantaloupes at 5 mg/mL concentration [11] and *Listeria* sp. by 2 log CFU/cm^2 on chicken skin at 0.75 % of shell extract (proprietary solvent free extraction system) [12]. In these studies, similar reductions were achieved at a lower concentration which could be due to direct application of just the extract and the proprietary extraction process that maximizes the antimicrobial contents [12]. Method of extraction have shown to be a factor affecting the antimicrobial efficacy of pecan shell extracts [7, 11], thus, various extraction methods need to be explored for optimum extraction of antimicrobial compounds from shells to achieve higher antimicrobial property.

There was no significant difference in *S. enterica* survival between control, P or PSE-P coated blueberries immediately after treatment and during storage. *S. enterica* decreased from 3.75 to 3.99 on day 0–3.04–3.37 log CFU/g by day 15 on all three samples. Likewise, no significant difference in survival of *S. aureus* was observed between water washed (control) and PSE-P coated blueberries, except on day 5 where higher ($P < 0.05$) counts were seen in PSE-P. Notably, higher ($P < 0.05$) survival of *S. aureus* was observed in

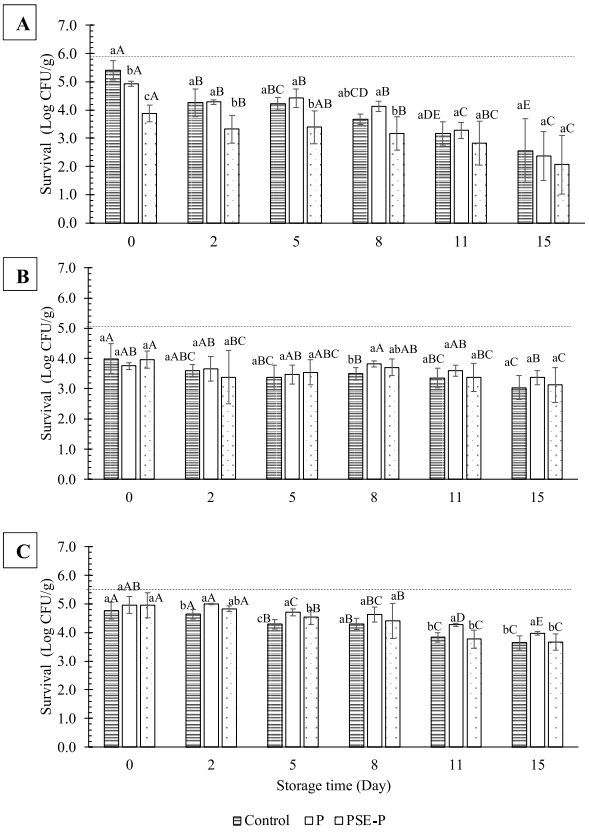

Fig. 1. Survival of *Listeria monocytogenes* (A), *Salmonella enterica* (B) and, *Staphylococcus aureus* (C) on pecan shell extract-pullulan coated blueberries during 15 days refrigerated storage. Mean bars with different small letters within treatments on each day and different capital letters for each treatment over days are significantly different ($P < 0.05$). Bacterial load post-inoculation but before treatment (Day 0) on blueberries were 5.94 ± 0.07 Log CFU/g (*Listeria monocytogenes*), 5.05 ± 0.26 Log CFU/g (*Salmonella enterica*) and 5.56 ± 0.48 Log CFU/g (*Staphylococcus aureus*) and is represented with dotted straight line in the graph. Treatments: Control = water, P = 5 % pullulan solution, PSE-P = 5 % aqueous pecan shell extract+5 % pullulan.

pullulan-only (P) coated blueberries (4.96–3.97 log CFU/g) than control (4.77–3.64 log CFU/g) and PSE-P (4.96–3.67 log CFU/g) by day 15. Pullulan itself has no antimicrobial property of its own, furthermore, it is hydrophilic in nature [13] due to which it attracts water to the coated surface. Thus, when exposed to humid environment during storage it could provide favorable moist condition for bacteria to survive on the surface. This further suggests that although shell extracts have antimicrobial property, pullulan may create favorable condition for the survival or growth of bacterial pathogens when used as a coating for the extracts.

The disc-diffusion study showed that pullulan coating incorporated with pecan shell extracts were effective against *S. aureus* and *S. enterica* (Table 1), but during the *in vivo* study on blueberry no significant effect of extract was observed. Several studies have observed this phenomenon of decreasing efficacy of bioactive compounds from *in vitro* to *in vivo* owing to slow and selective movement of compounds from coating to the fruit surface thus reducing its effectiveness during storage [34,35]. Studies have shown that the antimicrobial efficacy of pecan shell extract are also pathogen and strain specific [7,11] thus, different strains of bacteria used in the antimicrobial coating study which was not used during the disc-diffusion antimicrobial screening study may have been the reason for organism's resistance towards the shell extracts. Our results suggest that aqueous 5 % pecan shell extract-pullulan coating as a post-harvest treatment on blueberries has potential to reduce *L. monocytogenes* by 2 log CFU/g immediately upon treatment and lower its survival during storage. However, there is no significant effect against *S. enterica* and *S. aureus* at tested concentrations. For higher microbial inactivation, optimizing extraction process, integrating the extracts with other antimicrobial substances and/or coating medium possessing antimicrobial properties [36] could be beneficial which needs to be further explored.

3.3. Effect of pecan shell extract-pullulan coating on color, pH, TSS, and texture

Pecan shell extract-pullulan coating's impact on the color of coated blueberries is presented in Table 2. PSE-P had no significant effect on the L* (lightness) and a* (redness) (except on day 0); but significant effect ($P < 0.05$) on b* (blueness) of blueberries. Storage time had no significant effect on L*, a* and b* of C, P or PSE-P coated blueberries. On day 0, immediately after coating, PSE-P had higher ($P < 0.05$) a* value (0.81 ± 0.24) than control and P coated blueberries (0.42–0.59) indicating that extract coated blueberries had slightly red tinge on the surface. Pecan shell extract are red in color due to the presence of phlobaphenes, a condensed tannin [37], which could be the reason for red coloration in extracts and coated produce. According to International Commission on Illumination (CIE) L*a*b* color chart, the -b* values closer to 0 indicates samples are less blue in color [38]. The b* (blueness) values for the PSE-P (−0.80 ± 0.52) coated blueberries were significantly different and closer to zero than control (−2.40 ± 0.97) or P (−1.62 ± 0.46) immediately after coating on day 0 and throughout the study. This suggests that PSE-P blueberries were less bluish in color which could be due to the brown-red color of the coating material. Aqueous pecan shell extract treated cantaloupe samples and catfish filet samples had higher a* (redness) values but were comparable to that of control [11]. Although the b* (blueness) of coated blueberries were impacted due to the shell extract in this study; overall, ΔE showed that visually, the color were similar ($P > 0.05$) and comparable to the control (Table 2) throughout the study. The results indicated that PSE-P coating treatment and storage time showed no significant effect on the color of coated blueberry.

Table 2
Color of pecan shell extract-pullulan coated blueberries during 15 days of refrigerated storage.

Samples	Storage Days					
	0	2	5	8	11	15
L*						
Control	$33.87 \pm 3.22_{aAB}$	$33.49 \pm 0.79_{aAB}$	$34.23 \pm 0.60_{aA}$	$34.23 \pm 0.72_{aAB}$	$31.91 \pm 0.94_{aB}$	$33.9 \pm 2.34_{aAB}$
P	$31.54 \pm 1.68_{aB}$	$32.77 \pm 2.32_{aAB}$	$33.23 \pm 0.13_{aAB}$	$32.25 \pm 0.26_{aAB}$	$32.22 \pm 1.34_{aB}$	$34.52 \pm 2.72_{aA}$
PSE-P	$32.27 \pm 1.31_{aA}$	$32.54 \pm 1.73_{aA}$	$33.07 \pm 2.56_{aA}$	$32 \pm 1.38_{aA}$	$32.15 \pm 1.31_{aA}$	$32.86 \pm 0.61_{aA}$
a*						
Control	$0.42 \pm 0.10_{bA}$	$0.52 \pm 0.02_{aA}$	$0.46 \pm 0.04_{aA}$	$1.15 \pm 0.85_{aA}$	$0.47 \pm 0.06_{aA}$	$0.40 \pm 0.19_{aA}$
P	$0.59 \pm 0.07_{abA}$	$0.46 \pm 0.00_{aAB}$	$0.88 \pm 0.27_{aA}$	$0.55 \pm 0.32_{aAB}$	$0.49 \pm 0.12_{aAB}$	$0.34 \pm 0.03_{aB}$
PSE-P	$0.81 \pm 0.24_{aA}$	$0.51 \pm 0.01_{aB}$	$0.96 \pm 0.17_{aAB}$	$0.71 \pm 0.33_{aAB}$	$0.53 \pm 0.13_{aB}$	$0.54 \pm 0.13_{aB}$
b*						
Control	$-2.40 \pm 0.97_{bA}$	$-2.13 \pm 0.01_{bA}$	$-1.81 \pm 0.17_{bA}$	$-1.92 \pm 0.11_{bA}$	$-1.73 \pm 0.22_{abA}$	$-2.00 \pm 0.99_{bA}$
P	$-1.62 \pm 0.46_{bA}$	$-1.83 \pm 0.46_{bA}$	$-1.55 \pm 0.48_{abA}$	$-1.72 \pm 0.11_{abA}$	$-2.81 \pm 1.19_{bB}$	$-2.19 \pm 1.11_{bAB}$
PSE-P	$-0.80 \pm 0.52_{aA}$	$-1.04 \pm 0.17_{aA}$	$-0.95 \pm 0.29_{aA}$	$-0.89 \pm 0.10_{aA}$	$-1.43 \pm 0.39_{aA}$	$-0.975 \pm 0.15_{aA}$
[a]ΔE						
Control	$0.00 \pm 0.00_{a}$	$1.86 \pm 0.62_{a}$	$2.05 \pm 0.24_{a}$	$2.16 \pm 0.55_{a}$	$2.14 \pm 2.47_{a}$	$0.77 \pm 0.04_{b}$
P	$3.63 \pm 3.44_{a}$	$4.06 \pm 1.69_{a}$	$2.51 \pm 1.30_{a}$	$2.29 \pm 2.32_{a}$	$2.92 \pm 0.17_{a}$	$4.47 \pm 0.77_{a}$
PSE-P	$2.49 \pm 1.47_{a}$	$1.95 \pm 1.56_{a}$	$1.76 \pm 0.89_{a}$	$2.44 \pm 2.11_{a}$	$2.04 \pm 1.87_{a}$	$2.34 \pm 1.62_{ab}$

Mean values with different lower-case and upper-case letters within same column and row, respectively, are significantly different ($P < 0.05$) for each color parameter.
[a] Mean values with different lower-case letters within the same column are significantly different ($P < 0.05$).
[a] Color difference (ΔE) is measured against the control on 0 d as reference.
Treatments: Control = water, P = 5 % pullulan solution, PSE-P = 5 % aqueous pecan shell extract+5 % pullulan.

The effect of coating treatment (PSE-P, P, and control) on the pH and TSS of blueberries is shown in Table 3. TSS (%) of blueberries did not change ($P > 0.05$) throughout the experiment, regardless of the treatment, as shown in Table 3. PSE-P coating did not show significant effect on the pH of blueberries, except on day 0 (immediately after coating) and day 5. Similar findings were reported by Pobiega et al. (2020) where brush application of pullulan with propolis on blueberries showed no effect on its TSS (Total Soluble Solids), and pH during 21 days storage [17].

Fruit firmness is a major quality and shelf-life indicator for blueberries. The texture (firmness) of blueberries as affected by the coating treatments are shown in Table 3. On day 0, the firmness of PSE-P and control blueberries were similar but pullulan-only coating reduced ($P < 0.05$) the firmness than control. During storage, the firmness for control, P and PSE-P blueberries were similar ($P > 0.05$). But by the end of storage (day 15), the firmness values for PSE-P coated blueberries (7.57 ± 1.94) were higher ($P < 0.05$) than P (5.12 ± 2.22) and control (5.53 ± 2.87). Similar trends were observed where pullulan coating showed significant decrease of firmness (~30 %) in strawberries, comparable to control, as opposed to only 12 % loss due to cinnamon essential oil nano emulsion pullulan coating [18]. Pullulan does not provide good water barrier property due to its high affinity towards water thus it cannot stay intact on coated substance, fails to cover their surface, and does not exhibit good moisture barrier property [39] which could be the reason for its loss of firmness. Addition of hydrophobic compounds like pecan shell extracts can reduce the permeability [18] thus, help in maintaining the firmness during storage. Overall, PSE-P coating showed no significant impact on the color, pH and TSS of blueberries and further helped in upholding its texture throughout storage.

3.4. Effect of pecan shell extract-pullulan coating on native microbial population

The effect of pecan shell extract-pullulan coating on its native microbial population i.e., on aerobic count and yeast and mold count are presented in Fig. 2. Pullulan coating by itself or control did not show any effect on reduction of native microbial population of blueberries. On day 0, the PSE-P coating significantly reduced the native bacterial population on blueberries by 0.7 log CFU/g than when washed with water (control) or coated with pullulan-only (P). During 15 days refrigerated storage, there was no significant difference in aerobic plate counts between control, P or PSE-P coated blueberries. The aerobic counts of control, P and PSE-P coated blueberries were 2.74, 2.47 and 2.52 log CFU/g, respectively by day 15. Similar to the results observed on *in vitro* antifungal study (Table 1), there was no significant effect of the treatment (control, P, or PSE-P) on the yeast and mold count of blueberries throughout storage. Caxambu et al. (2016) showed that aqueous pecan shell extracts applied on lettuce leaves and stored for 5 days at refrigerated conditions had no effect on its yeast population but significantly reduced the natural microbial population (mesophilic and psychrotrophic organism) by 2.5–4.2 log CFU/g [10]. In our study, we used a lower volume of coating solution (0.016 mL of 5 % PSE-P coating solution/g blueberry) compared to previous study, that directly applied 1 mL of 1 % aqueous pecan shell extract on 1 g of lettuce leaves [10]. Thus, reduced extract concentration could be responsible for the observed lower antimicrobial effect in this study. Similarly, Babu et al. (2013) showed that pecan shell extracts at 0.75 % significantly reduced the native microbial population of chicken skin by 4.4 log which could be due to solvent-free extraction method maximizing the concentration of antimicrobials [12]. Although PSE-P showed promise in reducing native microbial population immediately after coating, no significant effect was observed during storage. Higher concentrations of extract or optimizing the extraction process to maximize the antimicrobials could be beneficial in reducing the native microbial population and extending the shelf-life.

3.5. Effect of pecan shell extract-pullulan coating on spoilage rate and weight loss

Spoilage rate and weight loss of coated blueberries when stored at 4 °C for 15 days is presented in Fig. 3. Signs of spoilage were visible from day 8 onwards for all samples with significantly higher spoilage rate and weight loss observed for pullulan coated samples,

Table 3
Total soluble solids (TSS), pH, and texture of pecan shell extract-pullulan coated blueberries during 15 days of refrigerated storage.

Samples	Storage Days					
	0	2	5	8	11	15
TSS (%)						
Control	$12.03 \pm 1.20_{aAB}$	$11.95 \pm 0.77_{aA}$	$11.55 \pm 0.62_{aB}$	$12.73 \pm 0.86_{aAB}$	$11.90 \pm 1.21_{aAB}$	$12.85 \pm 0.47_{aAB}$
P	$12.78 \pm 0.39_{aA}$	$11.28 \pm 0.26_{aB}$	$11.42 \pm 0.50_{aB}$	$13.00 \pm 1.56_{aA}$	$12.20 \pm 0.42_{aAB}$	$12.98 \pm 1.71_{aA}$
PSE-P	$12.25 \pm 0.54_{aAB}$	$11.80 \pm 0.58_{aB}$	$11.65 \pm 0.26_{aB}$	$13.18 \pm 0.74_{aA}$	$12.08 \pm 0.15_{aAB}$	$12.89 \pm 0.25_{aAB}$
pH						
Control	$3.31 \pm 0.03_{aA}$	$3.12 \pm 0.05_{aB}$	$3.03 \pm 0.04_{aCD}$	$2.90 \pm 0.13_{abD}$	$3.10 \pm 0.15_{aBC}$	$3.00 \pm 0.08_{aCD}$
P	$3.11 \pm 0.14_{bAB}$	$3.13 \pm 0.05_{aA}$	$2.98 \pm 0.05_{abCD}$	$2.81 \pm 0.07_{bE}$	$2.92 \pm 0.07_{bDE}$	$3.04 \pm 0.04_{aBC}$
PSE-P	$3.13 \pm 0.02_{bA}$	$3.08 \pm 0.02_{aA}$	$2.93 \pm 0.03_{bB}$	$3.01 \pm 0.16_{aAB}$	$3.03 \pm 0.07_{abAB}$	$2.97 \pm 0.05_{aB}$
Texture (N)						
Control	$9.41 \pm 1.93_{aA}$	$10.03 \pm 1.37_{aA}$	$9.94 \pm 2.19_{aA}$	$8.81 \pm 3.35_{aA}$	$9.50 \pm 2.04_{aA}$	$5.53 \pm 2.87_{bB}$
P	$7.72 \pm 2.05_{bB}$	$8.29 \pm 2.06_{bAB}$	$9.61 \pm 2.54_{aA}$	$8.65 \pm 3.46_{aAB}$	$9.28 \pm 1.63_{aA}$	$5.12 \pm 2.22_{bC}$
PSE-P	$8.82 \pm 2.08_{abBC}$	$8.42 \pm 1.88_{bBC}$	$10.43 \pm 1.82_{aA}$	$9.21 \pm 1.87_{aABC}$	$9.53 \pm 2.41_{aAB}$	$7.57 \pm 1.94_{aC}$

Mean values with different lower-case and upper-case letters within same column and row, respectively, are significantly different ($P < 0.05$) for each test parameter.
Treatments: Control = water, P = 5 % pullulan solution, PSE-P = 5 % aqueous pecan shell extract+5 % pullulan.

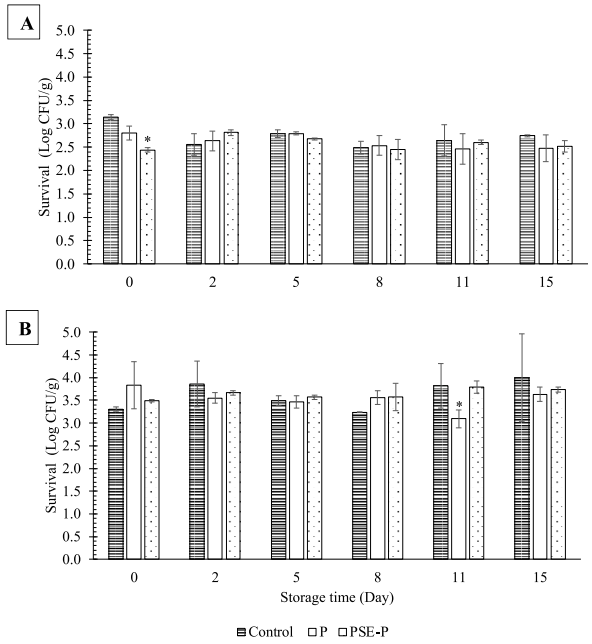

Fig. 2. Survival of native microbial population (aerobic count) (A) and, yeast and mold (B) on pecan shell extract-pullulan coated blueberries during 15 days refrigerated storage. Mean bars with * represents significant difference ($P < 0.05$) from control on the same day. Treatments: Control = water, P = 5 % pullulan solution, PSE-P = 5 % aqueous pecan shell extract+5 % pullulan.

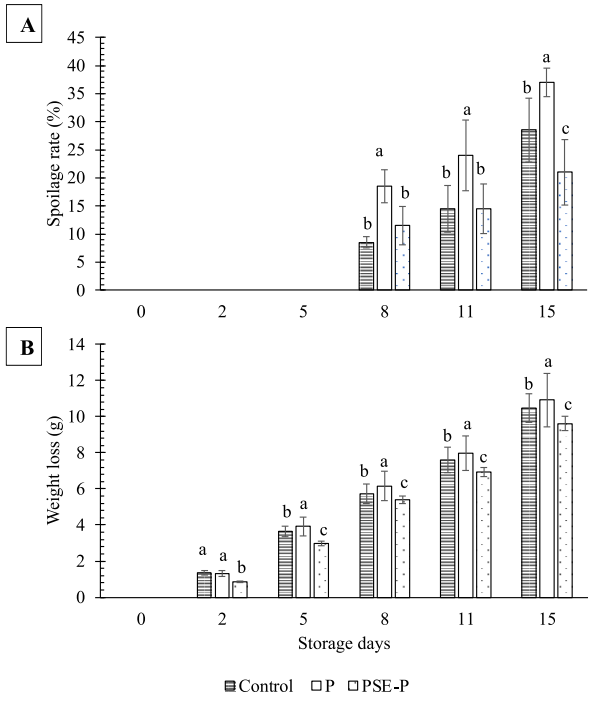

Fig. 3. Spoilage rate (A) and weight loss (B) of pecan shell extract-pullulan coated blueberries during 15 days refrigerated storage. Mean bars with different small letters within treatments on each day are significantly different ($P < 0.05$). Treatments: Control = water, P = 5 % pullulan solution, PSE-P = 5 % aqueous pecan shell extract+5 % pullulan.

followed by control and least for PSE-P coated until day 15. Control and pullulan coated blueberries showed shrinking appearance day 8 onwards whereas, PSE-P coated blueberries retained, comparatively, good appearance until day 15 (Supplemental Table 1). On day 15, blueberries that were PSE-P coated had significantly lower (21 %) spoilage rate than control (28.5 %) and P-coated (37 %).

Likewise, PSE-P coated blueberries showed significantly lower weight loss (9.61 %) than control (10.46 %) and pullulan coated (10.91 %). Blueberries, under ideal storage conditions of 31–32 °F and 90–95 % relative humidity, typically have a shelf-life of 10–18 days [40]. The application of PSE-P coating has shown promise in prolonging this shelf-life, as evidenced by a lower spoilage rate and reduced weight loss observed until day 15 of refrigerated storage compared to uncoated or pullulan only coated blueberries.

4. Conclusions

This study evaluated the effect of pecan shell extracts-pullulan against several food borne pathogens *in vitro* and its effect as an antimicrobial coating on safety and quality of blueberries. Aqueous and ethanolic pecan shell extracts incorporated into pullulan film showed antimicrobial property at 5 and 10 % concentrations however, no antifungal property was observed. Higher inhibition was observed for Gram positive bacteria than Gram negative. The antimicrobial efficacy of pecan shell extract depended on extraction methods and extract concentration. PSE-P coating reduced *L. monocytogenes* by 2 log on blueberries immediately after coating and showed gradual decrease in growth during 15 days refrigerated storage but, no significant effect was observed against *S. enterica* and *S. aureus.* The pecan shell extract-pullulan coating did not affect color, TSS and pH of blueberries during 15 days refrigerated storage. Instead, PSE-P maintained the texture and significantly reduced the weight loss and spoilage rate in coated blueberries until the end of study as compared to control. Thus, pecan shell extract could be a potential natural antimicrobial for application in food. Further studies are needed to better understand its antimicrobial mechanism, its application on other food matrices/packaging, optimize extraction process and explore alternative coating materials and/or antimicrobial compounds to incorporate into for enhanced bacterial inactivation. Consumer perception, particularly, acceptance of blueberries coated with pecan shell extract-pullulan also needs to be investigated.

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Data availability statement

Data will be made available upon request.

CRediT authorship contribution statement

Karuna Kharel: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Karolina Kraśniewska:** Writing – review & editing, Methodology. **Małgorzata Gniewosz:** Writing – review & editing, Methodology. **Witoon Prinyawiwatkul:** Writing – review & editing, Methodology. **Kathryn Fontenot:** Writing – review & editing, Methodology. **Achyut Adhikari:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Achyut Adhikari reports financial support was provided by USDA and Polish National Agency for Academic Exchange. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29610.

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