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Effect of pecan variety and the method of extraction on the antimicrobial activity of pecan shell extracts against different foodborne pathogens and their efficacy on food matrices

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26 **Abstract**

27 The shells of pecans are a rich source of bioactive compounds with potential inhibitory activity against

28 various pathogenic microorganisms. This study investigated the antimicrobial activity of pecan shell

29 extracts as effected by the type of cultivar and the method of extraction against various foodborne

30 bacterial pathogens. Defatted shell powders of 19 different pecan cultivars were subjected to aqueous and

31 ethanolic extraction (1:20 w/v) procedures, respectively. The minimum inhibitory concentration (MIC)

32 and minimum bactericidal concentration (MBC) of lyophilized pecan shell extracts dissolved in deionized

33 water containing 5% DMSO (v/v) were determined against multiple strains of *Listeria monocytogenes*,

34 *Salmonella enterica,* and *Escherichia coli* O157:H7. The antimicrobial activity of pecan shell extracts

35 was found to be pathogen specific and strain dependent. Overall, *L. monocytogenes* was found to be least

36 resistant to treatment with pecan shell extracts with an MIC and/or MBC values ranging from 1.25 to 5

37 mg/mL followed by *Salmonella enterica* (2.5 to ≥5 mg/mL) and *E. coli* O157:H7 (≥5 mg/mL). Type of

38 cultivar and the method of extraction found to have a variable effect on the antimicrobial activity.

39 Furthermore, the challenge studies on fresh-cut cantaloupes and thawed catfish fillets treated with 5

40 mg/mL pecan shell extracts and stored at 4ºC for up to 5 days showed a <0.5 to 4 log less growth in *L.*

41 *monocytogenes* when compared to the controls with no treatment. No significant change in the color

42 quality of treated food samples was observed with pecan shell extract treatment. The results of this study

43 showed promise to use pecan shell extracts as a natural antimicrobial agent to inhibit the growth of tested

44 foodborne bacterial pathogens.

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48 *Keywords*: Pecan; Shell; Cultivar; Extraction; Antimicrobial activity

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1. Introduction

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53   Microbiological contamination of foods with various foodborne bacterial pathogens is a major

54 concern to both consumers and the food industry alike. It was estimated that an approximate 9.4 million

55 illnesses are attributed to foodborne pathogens incurring $14 billion in losses annually in the United

56 States (Hoffmann et al., 2012). Some food categories have standardized protocols to either control or

57 reduce contamination during processing (Kumar, Shafiq, & Yousuf, 2015). However, these food products

58 can withstand processes such as high temperatures or pressures without greatly damaging the product.

59 Minimally processed products, however, lack efficient kill steps to control the pathogens (Bortolossi et

60 al., 2016). Foodborne pathogens such as *Salmonella* spp*., Listeria monocytogenes,* and *Escherichia coli*

61 O157:H7 have been a frequent problem in these products. This has led to the exploration of novel

62 antimicrobial compounds (Han, 2013). The antimicrobials being used may either inhibit or kill bacteria

63 and must always be Generally Recognized As Safe (GRAS) (Sung et al., 2013, Rhim, Park & Ha, 2013).

64 Considering this there has been an increase in interest for the use of natural antimicrobials (Rhim, Park &

65 Ha, 2013). The use of natural antimicrobial agents allows food processors to still maintain a minimally

66 processed status for their products and depending on the source of the antimicrobial, it may also be a

67 cheaper option than synthetic antimicrobials (Otoni et al., 2016, Irkin & Esmer, 2015). Thus, various

68 plant bioactive compounds are gaining attention as potential natural sources of food preservatives.

69   Pecan [*Carya illinoinensis* (Wangenh) C. Koch], a species of hickory tree native to North

70 America, is commercially cultivated in 14 states of the US for its edible seed (nut). The major production

71 states (Georgia, New Mexico, Louisiana, and Texas) account for approximately 75% of the total

72 production (NASS, 2018). The pecan crop is highly valued but greatly underutilized. In 2017, more than

73 270 million pounds of pecan nuts were cultivated in the United States, valued at over 500 million dollars

74 (NASS, 2018). Following harvest, over 90% of pecan nuts are processed to remove the outer shell layer,

75 and only the edible kernel is sold for consumption (NASS, 2018). Depending on the specific cultivar,

76 around 50% of the harvested pecan mass is shell weight (Worley, 1994). As it stands, they provide very

77 little to no revenue for pecan shellers and can be a significant disposal issue.

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78   Several studies have shown that pecan shells possess phenolic compounds ranging from phenolic

79   acids, flavan-3-ols, to anthocyanins that have antimicrobial and antioxidant properties (Villarreal-Lozoya

80   et al., 2007; Prado et al., 2014; Rosa et al., 2014). These compounds have been isolated from their natural

81   shell matrix by different extraction methods and have shown efficacy against various microorganisms

82   (Babu et al., 2013; Caxambu et al., 2016). The antimicrobial and antioxidant properties of pecan shell

83   extracts are found to be varied by factors such as method of extraction (Prado et al., 2014) and cultivar or

84   variety (Prado et al., 2009; Villarreal-Lozoya et al, 2007), growing region (Rosa, Alvarez-Parrilla, &

85   Shahidi, 2011; Rosa et al., 2014), cultivation method (Malik et al., 2009), and harvest year (Prado et al.,

86   2013). As it stands, there is a lack of studies that extensively compare the effect of pecan cultivar across a

87   large population while controlling the harvest year, growing region, and cultivation method. Furthermore,

88   the effect extraction method to obtain extracts with the highest potency across a range of pecan cultivars

89   has not been studied. Thus, the objectives of this study are to determine: (i) the effect of cultivar and

90   extraction method on the antimicrobial efficacy of pecan shell extracts obtained from pecans grown in the

91   Southern United States using the same cultivation methods and harvested in the same year against various

92   foodborne bacterial pathogens, and (ii) the antimicrobial efficacy of pecan shell extracts when tested on

93   real food matrices.

94   **2. Materials and methods**

95   *2.1. Selection of pecans*

96   A total of 19 different cultivars of in-shell pecans (Table. 1) harvested from several Louisiana

97   orchards during the October/November season of 2016-2017 were obtained from Louisiana State

98   University Agricultural Center's Pecan Research and Extension Station located in Bossier City, LA.

99   These pecans were stored in woven polypropylene mesh bags at 4°C until further use in the experiments.

100   *2.2. Preparation of pecan shell extracts*

101   *2.2.1. Sample preparation*

102   The shells of pecans were separated from the inner cuticle and kernel using a nutcracker. The

103   separated shells were crushed into small pieces using a pestle and mortar and dried in a hot air oven

104   (Model 1370 GM, VWR, Radnor, PA, USA) at 40ºC for 8 h. The dried shell pieces were further ground

105   into a fine powder (≤106 µm) that can pass through No. 140 size sieve. The resultant powders of 19 pecan

106   varieties were stored in amber bottles in darkness at -20ºC until further use.

107   *2.2.2. Defatting of samples*

108   An aliquot of 8 g powdered sample with 160 mL of hexane 1:20 (w/v) was taken into a 250 mL

109   amber bottle with a cap. The samples were then constantly mixed in a shaker incubator (Model C25KC,

110   New Brunswick Scientific, Edison, NJ, USA) at 160 rpm and 23ºC for 45 min. After the incubation, the

111   hexane fraction from the samples was filtered out under vacuum using Whatman® No. 1 filter paper. The

112   leftover powdered residue was put back into the same amber bottle and the defatting with hexane was

113   repeated two more times. After three cycles of fat extraction, the defatted residue was air-dried at room

114   temperature (23ºC) in a chemical hood for about 4 h in the dark to remove remaining hexane by

115   evaporation. Later, the defatted dried powder samples of different pecan varieties were stored at -20°C.

116   *2.2.3. Extraction of shell compounds*

117   Two methods of shell extraction procedures namely: (i). Aqueous and (ii). Ethanolic extractions

118   were followed in this study. Briefly, the procedure includes first 8 g of defatted pecan shell powder added

119   into 160 mL of boiling distilled water (1:20 w/v) in a 250 mL amber bottle and heated by maintaining the

120   temperature at 98±3ºC for 30 min in a hot water bath. Later, the contents of the bottle were cooled down

121   to room temperature and the extracts were filtered through Whatman® No.1 filter paper. Shortly after, the

122   liquid extracts were lyophilized using a Genesis Pilot Freeze Dryer (VirTis™, SP Scientific, Warminster,

123   PA, USA). In this manner, dry aqueous shell extraction powders of 19 pecan cultivars were prepared and

124   stored in amber centrifuge tubes in dark at 4ºC until further use. While the samples of defatted pecan shell

125   powders were added to ethanol (1:20 w/v) and incubated at 160 rpm for 1 h to obtain ethanolic

126   extractions. The extracts were filtered through Whatman® No.1 filter paper into a 250 ml amber bottles

127   and flushed with N_2 gas to remove air. The extracts were then lyophilized using a benchtop freeze dryer

128   (Thermo Savant Modulyod®-115, Thermo Scientific, Waltham, MA, USA) and stored at -20ºC until

129 further use. In this way, a total of 38 shell extract powders (19 aqueous + 19 ethanolic) were prepared to

130 test their antimicrobial activity.

131 *2.3. Selection of bacterial strains and inoculum preparation*

132   Multiple strains of *E. coli* O157:H7 (CDC 658, Cantaloupe outbreak strain; H1730, Lettuce

133 outbreak strain; W411, Pecan field isolate; Cocktail mixture of previous three), *Salmonella enterica*

134 (Anatum 1715, isolated from almond survey; Enteritidis PT 30, Raw almonds associated outbreak;

135 Tennessee K4643, Peanut butter associated outbreak; Cocktail mixture of previous three), and *Listeria*

136 *monocytogenes* (101M (serotype 4b), Beef associated outbreak; Scott A (serotype 4b) & V7 (serotype

137 1/2a), Milk associated outbreaks; LCDC 81-861 (serotype 4b); Raw cabbage associated outbreak;

138 Cocktail mixture of previous four) were tested in this study. All the strains were stored at -80°C in tryptic

139 soy broth (TSB) (Difco, Becton Dickinson, Sparks, MD, USA) containing 30% glycerol. Prior to each

140 experiment, the frozen cultures were activated by three successive passages by growing them overnight in

141 10 mL of TSB for *E. coli* O157:H7 and *S. enterica* and TSB with 0.6% yeast extract (TSBY) for *L.*

142 *monocytogenes* at 37°C. After that, each individual bacterial strain was cultured separately in 10 mL of

143 TSB or TSBY and incubated at 37°C for 16 h. Following the incubation, the cells were harvested at 5000

144 x g for 5 min. The resultant supernatant was decanted, and the pellet was re-suspended in 10 mL of sterile

145 phosphate-buffered saline (PBS, pH 7.2). This procedure was repeated twice, and the final pellets of

146 individual strains were re-suspended separately in 10 mL of PBS. Appropriate serial dilutions of the

147 individual strains were prepared in PBS to achieve a cell concentration of approximately 10^7 CFU/mL.

148 An equal volume of each strain suspension was combined to obtain a cocktail mixture of an individual

149 organism. Cell concentration was adjusted by measuring the absorbance at 600 nm using UV/Vis

150 spectrophotometer and confirmed by plating 100 μL portions of appropriate serial dilutions on tryptic soy

151 agar (TSA) (Difco Laboratories) plates and incubation at 37°C for 24 ± 2 h.

152 *2.4. Determination of MIC and MBC*

153   The MIC and MBC of the aqueous and ethanolic extracts were determined based on the Clinical

154 and Laboratory Standards Institute (CLSI) microdilution method (CLSI 2009) with some modifications

155 based on Prado et al. (2014) and Yuan et al. (2017). Briefly, a known weight of aqueous and/or ethanolic

156 extract powders was first reconstituted with deionized (DI) water containing 5% dimethylsulphoxide

157 (DMSO) to a concentration of about 10 mg/mL. Using a 96-well plate, the extracts were three-fold

158 serially diluted in TSB or TSBY, and 100 µL of the diluted extract was mixed with 100 µL respective

159 strains of bacterial inoculum and their cocktail mixtures to achieve a final concentration of extracts at

160 1.25, 2.5 and 5 mg/mL, and an inoculum concentration of 10^5 CFU/mL. The wells containing TSB with

161 100 µL inoculum and 100 µL gallic acid (1.25 to 5 mg/mL) or DI water with 5% DMSO were included as

162 a positive and negative control, respectively. The plates were sealed and incubated at 37ºC for 24 h. The

163 MIC of the samples was determined by visually observing no growth wells of the 96-well plate. Further

164 the viability of cells was confirmed by adding 40 µL of aqueous solution of 2, 3, 5-triphenyltetrazolium

165 chloride (INT) 0.5% (m/v) dye and incubation at 37ºC for 1 h. The viable bacterial cells reduced the

166 yellow colored dye and turned into a pink color for better visual observation to determine MICs. The MIC

167 was defined as the lowest extract concentration that prevented the color change of the medium and

168 exhibited complete inhibition of microbial growth. From each well of the microplate that showed no

169 visible growth and/or color change, a 50 µL aliquots were pour plated onto either Sorbitol MacConkey

170 (SMAC) agar (Criterion, Hardy Diagnostics, Santa Maria, CA) for culturing *E. coli* O157:H7 or Xylose

171 Lysine Deoxycholate (XLD) agar (Criterion, Hardy Diagnostics, Santa Maria, CA) for culturing *S.*

172 *enterica* or Oxford agar (Difco, Becton Dickinson, Sparks, MD) for culturing *L.* monocytogenes,

173 respectively. The plates were incubated at 37ºC for 24 h. After the incubation, the plates with ≤3 visible

174 colonies were regarded as minimum bactericidal concentrations (MBC) in this study.

175 *2.5. Testing on catfish fillets and/or fresh-cut cantaloupes against L. monocytogenes*

176 Fresh frozen catfish fillets and whole cantaloupes were purchased from the local supermarket and

177 stored at -20ºC and 4ºC, respectively until use. Prior to each experiment, the frozen catfish samples were

178 thawed at 4ºC overnight. These thawed samples were aseptically cut into 4 x 4 cm^2 size and about 5±0.5 g

179 weight using a sterile knife. Similarly, the whole cantaloupe rinds were cored off using a sterile peeler

180 and the edible fruit was cut into several 4 x 4 cm^2 (weight 5±0.5 g) sample sizes. Later, the cut catfish and

181 cantaloupe samples were transferred into sterile petri dishes for treatment with aqueous and ethanolic

182 pecan shell extracts of Cuddo and Nacono cultivars. These varieties were selected as a model extracts

183 based on MIC and MBC test results. Briefly, the treatment of sample(s) involved a 50 µL of inoculum

184 consisted of a cocktail of 4 strains was first spread on one side of the sample and dried for 30 min in a

185 biosafety cabinet. The samples were then flipped around using sterile forceps and inoculated the other

186 side with another 50 µL of inoculum to achieve final microbial inoculum concentration of about 10^6

187 CFU/sample. Each inoculated sample was then spread with 100 µL pecan shell extract (either Cuddo

188 and/or Nacono varieties) at 5 mg/mL concentration and allowed to air dry for 15 min inside the biosafety

189 cabinet. The same procedure of the treatment with shell extract was repeated on the other side of the food

190 samples. Deionized water with 5 % DMSO was used as a control treatment. The treated catfish samples

191 were individually packaged in a polyethylene film and stored at 4ºC. While the cantaloupe samples were

192 placed in a petri dish with a lid-on and stored at 4 ºC. The number of viable bacteria was enumerated on

193 0, 1, 3, and 5 days following the treatment by processing each sample in 25 mL of 0.1% peptone water

194 and pummeling in a Bagmixer® 400 blender (Interscience Laboratories Inc., MA, USA). Appropriate

195 serial dilutions were prepared and spread plated onto oxford agar and incubated at 37ºC for 24 h. Aerobic

196 plate count (APC) and yeast and mold counts were determined on non-inoculated treated and control

197 samples using Petrifilms^{TM} (3M^{TM} , St. Paul, MN) as per the manufacturer's guidelines. The results of

198 APC and yeast & mold show no significant difference between control and treatment samples.

199 *2.6. Color measurement*

200 Any changes in the color of non-inoculated fresh-cut cantaloupe and catfish fillets following the

201 treatment with the pecan shell extracts and storage at 4°C over a 5-day storage period was determined.

202 Color was measured using a spectrophotometer (CM-5, Konica Minolta, Inc., Ramsey, NJ, USA). The color

203 values L* (100=white, 0=black), a* (positive=redness, negative=greenness), b*(positive=yellow,

204 negative=blue) were measured for duplicate samples at three different locations on each sample.

205 *2.7. Statistical analysis*

206   Data were analyzed by the analysis of variance (ANOVA) procedure using the Statistical Analysis

207   System (SAS software Version 9.1, SAS Institute Inc., Cary, NC). T-tests were performed for pairwise

208   comparisons. The least significant difference of means tests was performed for multiple comparisons. All

209   tests were performed with a 0.05 level of significance.

210 **3. Results and Discussion**

212 3.1. Determination of MIC and MBC

214 *3.1.1. Effect of cultivar and type of bacterial pathogen/strains*

216   The MIC and MBCs of pecan shell extracts against different foodborne bacterial pathogens were

217   shown in Table 1. The MICs of different strains of *L. monocytogenes, Salmonella enterica* and *E. coli*

218   O157:H7 ranged from 1.25 to 5 mg/mL, 2.5 to 5 mg/mL and 5 mg/mL, respectively. While their

219   respective MBCs were either maintained at the same level as MICs (1.25 to 5 mg/mL) or increased

220   further from 2.5 to >5 mg/mL depending upon the type of pecan cultivar used for shell extracts and the

221   bacterial strain and/or strain-mixtures. For example, the MICs were found to be highest for the multi-

222   strain mixture of *L. monocytogenes* and strain 101M (5 mg/mL) followed by V7 or Scott-A (2.5 to 5

223   mg/mL), and LCDC 81-861 (1.25 mg/mL), respectively (Table 1). This shows that different strains of *L.*

224   *monocytogenes* showed varied susceptibility to pecan shell extracts. A study by Prado et al. (2014)

225   observed similar variation in the susceptibility of different *L. monocytogenes* strains to pecan shell extract

226   treatments. They reported MICs of 2.5 and 1.25 mg/mL for *L. monocytogenes* ATCC 19117 and ATCC

227   19112 strains, respectively. Similar trends were also observed for different strains of *Staphylococcus*

228   *aureus* (0.15 to 0.46 mg/mL). Another study by Babu et al. (2013) found that the individual strains and

229   serotypes of *L. monocytogenes* exhibited differences in their sensitivity to antimicrobial treatment with

230   roasted and unroasted organic pecan shell extracts. The MIC values reported in this study were ranged

231   from 0.188 to 6 %. They concluded that these differences were potentially attributed to the differences in

232   the organisms' genetic potential to withstand antimicrobial treatments (Lungu et al., 2011; Milillo et al.,

233   2012). On the other hand, the results of the current study indicate no difference in the MICs among

234   different strains of *E. coli* O157:H7 (Table 1). This shows that the susceptibility to pecan shell extract

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235 antimicrobial treatment is pathogen and strain-specific. Among the tested foodborne bacterial pathogens,

236 *E. coli* O157:H7 was found to be the most resistant to pecan shell extract treatment followed by

237 *Salmonella enterica* and *L. monocytogenes*, respectively. Furthermore, the pecan variety has shown to

238 influence the antimicrobial activity depending upon the susceptibility of bacterial strain and/or pathogen.

239 For example, cultivars Jackson, Desirable, Melrose, and Success found to have higher MICs (5 mg/mL)

240 against strain Scott-A of *L. monocytogenes* compared to other cultivars which showed an MIC of 2.5

241 mg/mL (Table 1). Likewise, cultivars Elliot, Caddo, Jackson, Desirable, Moreland, Melrose, Kiowa,

242 Success, Summer, Schley, and Pawnee showed an MIC of 5 mg/mL against strain V7 compared to other

243 cultivars which exhibited an MIC of 2.5 mg/mL (Table 1). Similar variations in the MIC/MBCs with the

244 type of cultivar were also observed in case of *Salmonella enterica* (Table 1). While no change in the

245 MICs of *L. monocytogenes* strains101M, CDC, 4-strain mixture and *E. coli* O157:H7 (Table 1) were

246 observed with respect to pecan cultivar. This variation in strain susceptibility to pecan shell extracts from

247 different cultivars can be attributed to: (1) genetic variations and susceptibility to antimicrobial treatment

248 among the tested bacterial strains, (2) the variations in the bio-actives composition across different

249 varieties of pecan shells. As per our knowledge, this is the first study that investigated the effect of

250 different pecan cultivars on antimicrobial activity. Our preliminary studies investigating bioactive profiles

251 of various pecan shell extracts indicate that the pecan variety has a significant effect on the content of

252 tannins. Follow-up studies should be conducted to determine variations in the bioactive composition

253 across different tested pecan cultivars and their potential effect on antimicrobial activity.

254 *3.1.2. Effect of method of extraction*

255   Table. 2 shows the effect of the method of extraction on the antimicrobial activity of pecan shell

256 extracts. Method of extraction found to have a significant effect on different strains of *L. monocytogenes*

257 and *Salmonella enterica*. For example, MICs of *L. monocytogenes* strains Scott-A and V7 were found to

258 be in the range of 2.5 to 5 mg/mL for aqueous pecan shell extractions. While the same strains had an MIC

259 of 1.25 to 5 mg/mL (Scott-A) and 2.5 mg/mL (V7) in case of ethanolic extractions. Whereas, no change

260 in the MICs were observed for CDC, 101M, 4-strain mixture of *L. monocytogenes* with the method of

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261 extraction. This reaffirms that the type of *L. monocytogenes* strain found to have a significant effect on

262 the MIC while ethanolic pecan shell extracts showed relatively lower MICs against certain strains

263 compared to aqueous extractions. However, the same trend was not observed in case of *Salmonella*

264 *enterica* where ethanolic extractions had higher (≥5mg/mL) MICs compared to aqueous extractions (2.5-5

265 mg/mL). No significant difference in MBCs was observed except strain PT30 of *S. enterica*. These results

266 corroborate that the method of shell extraction influence the antimicrobial activity and this effect is strain

267 and/or pathogen-specific. Prado et al. (2014) compared the effect of method of pecan shell extraction

268 (aqueous infusion, infusion followed by spray drying, ethanol extraction and supercritical extraction) on

269 the phenolics profile and antimicrobial activity against different bacteria. They reported that extract

270 obtained through infusion followed by spray drying was more effective at lower concentrations against

271 different strains of *L. monocytogenes* compared to the extracts obtained by just aqueous infusion and by

272 ethanol extraction. This is mainly attributed to the increased concentration of total phenolics and

273 condensed tannins in the extracts with the method of extraction. It should be noted that the Prado et al.

274 (2014) study conducted on a single pecan variety and not fully explained the effect of different pecan

275 varieties. The results of the current study not conclusively provided any evidence to fully understand the

276 effect of extraction method on the antimicrobial activity. However, the results indicate the interaction

277 effect of pecan variety, method of extraction, type of bacterial pathogen and strain. Further studies need to

278 be conducted to fully understand the correlation between the composition of extracts and antimicrobial

279 activity as influenced by the pecan variety and the method of extraction.

280 3.2. Antimicrobial efficacy of shell extracts on catfish fillets and fresh-cut cantaloupe

281   The antimicrobial efficacy of aqueous and ethanolic shell extracts of two selected varieties of

282 pecans (i.e. Caddo and Nacono), when tested against 4-strain mixture of *L. monocytogenes* on Catfish

283 fillets and Fresh-cut cantaloupes, were shown in Table 3. Catfish fillets when treated with pecan shell

284 extracts at 5 mg/mL concentration and stored at 4°C for up to 5 days found to reduce the growth of *L.*

285 *monocytogenes* for up to 3 days when compared to control samples with just sterile water + 5% DMSO

286 treatment. For example, when the catfish fillet samples inoculated with *L. monocytogenes* (4-strain*)* at

287   about 5 to 6 log CFU/sample and subjected to treatment with aqueous extract of Nacono variety a

288   reduction of 2.8±0.14, 3.97±0.21, 0.9±0.36, and 0.01±0.56 log CFU/sample was observed at the end of 0,

289   1, 3, and 5 days storage at 5°C when compared to controls (Table 3). A significant (P<0.05) difference in

290   the growth reductions was observed between Days 0, 1, and 3. While the difference is not statistically

291   significant between days 3 and 5. The highest reduction of 3.97 log CFU/sample was observed on Day-1,

292   and reductions were decreased thereafter to below 1 log CFU/sample at the end of 5-day storage period.

293   Similar reduction trends were also observed for other tested pecan shell extractions. No significant

294   difference in the log reductions was observed with respect to pecan variety and the method of extraction

295   among the tested samples (Table 3). On the other hand, a minimal effect of pecan shell extract treatment

296   in reducing the growth of *L. monocytogenes* was observed on fresh-cut cantaloupes (Table 3). At the end

297   of 5-days storage a reduction of only 1.15±0.02, 0.13±0.01, 1.22±1, and 0.64±0.5 log CFU/sample were

298   observed for different pecan shell extract samples when compared with the controls. No significant

299   difference in the reduction on fresh-cut cantaloupes was observed with pecan variety and method of

300   extraction. This variation can be attributed to the inherent differences in the physico-chemical properties

301   of tested food matrices. Higher extract concentrations beyond the minimum inhibitory concentrations of

302   tested pathogens need to be tested to overcome the food matrix interfering effect and achieve greater

303   overall reductions. Yuan et al. (2017) found that a high concentration (16 mg/mL) of *Cinnamomum*

304   *javanicum* extract is needed to inhibit or reduce the growth of *L. monocytogenes* growth on smoked

305   salmon in contrast to the observed MIC of 0.13 mg/mL. Another study by Kang & Song (2017) reported

306   that treatment of fresh produce such as red chard, beet, chicon, and red mustard leaves with pomegranate

307   pomace extract containing wash solution at 7 mg/mL showed a reduction of 2.88, 2.97, 2.25, and 1.96 log

308   CFU/g in the growth of *L. monocytogenes*, respectively. Similarly, Kim et al. (2011) reported a reduction

309   of 1-4 log CFU/g of foodborne pathogens on fresh lettuce when treated with clove extract at 10 to 100

310   mg/mL for 1 to 10 min. However, the method of treatment in these two studies is washing which is

311   different from the simple application of antimicrobial compounds followed in the current study. Thus, the

312   findings of the current study showed promise to use pecan shell extracts as natural antimicrobial agents on

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313   real food matrices. Follow-up studies were conducted to determine the effect of pecan shell extract

314   application on the color quality of tested food matrices.

315   3.3. Effect of pecan shell extract treatment on color

316   Color values of pecan shell extract treated catfish fillet and fresh-cut cantaloupe samples when

317   stored at 4°C for up to 5 days were presented in Table 4. No significant difference (P>0.05) in the

318   lightness (L*) was observed between treatment and control samples of catfish at the end of 5-day storage.

319   With few exceptions, most of the catfish samples treated with different pecan shell extract found to be

320   darker in color (i.e. lower L* value) compared to control samples after 1 and 3 days of storage. Whereas,

321   no significant difference in the greenness (a*) and blueness (b*) was observed between control and

322   treatment samples over a 5-days storage period except Caddo-aqueous extract treatment samples at

323   certain sampling times (Table 4). Almost all fresh-cut cantaloupe samples treated with pecan shell

324   extracts were found to be lighter in color (i.e. higher L* value) compared to untreated samples. The

325   redness (a*) of the fresh-cut cantaloupe samples was higher on the treated samples as compared with the

326   non-treated samples. However, in most cases, the difference is statistically not significant (P>0.05). The

327   yellowness (b*) on all fresh-cut cantaloupe samples increased after five days of storage. These results

328   indicate that the effect of pecan shell extract on the color quality is food matrix dependent and at the

329   tested concentration levels the color quality of tested foods is comparable to controls.

330   **4. Conclusion**

331   In this study, the antimicrobial activity of pecan shell extracts was tested against different foodborne

332   bacterial pathogens. The antimicrobial efficacy was found to be both pathogens and strain-specific.

333   Among the tested organisms, *L. monocytogenes* found to be more susceptible to pecan shell extract

334   treatment followed by *S. enterica* and *E. coli* O157:H7. Type of pecan cultivar and method of shell

335   extraction showed a variable effect on the MIC (1.25 to 5 mg/mL) and MBC values (1.25 to ≥5 mg/mL)

336   depending upon the type of pathogen and strain. Treatment of Catfish fillets and fresh-cut cantaloupes

337   with pecan shell extract reduced the growth of *L. monocytogenes* to a level of 0.5 to 4 log CFU/sample for

338   up to 5 days by maintaining comparable color quality as of control samples. The findings of this study

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339   showed promise of using pecan shell extracts as a potential natural antimicrobial agent to reduce the

340   growth of bacterial pathogens in food products. Further studies need to be conducted to better understand

341   the bioactive profile in various pecan cultivars and the effect on antimicrobial activity.

342   **Acknowledgments**

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444   extract against *Listeria monocytogenes* and its application potential with smoked salmon. *Int. J. of*
445   *Food Microbiology*, 260, 42-50.

2 Table 1. Effect of type of cultivar on minimum inhibitory concentration (MIC) & minimum bactericidal concentration (MBC) of aqueous pecan
3 shell extracts against different foodborne bacterial pathogens
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Pecan cultivar	*Listeria monocytogenes*																		*Salmonella* enterica										*E. coli* O157:H7[1]	
	V7		101M		CDC		Scott-A		4-strain		1715		PT30		K4693		3-strain		O157:H7[1]	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gloria Grande	2.5	5	5	5	1.25	1.25	2.5	2.5	5	5	2.5	5	2.5	2.5	2.5	>5	2.5	>5	5	>5
Elliot	5	5	5	5	1.25	1.25	2.5	5	5	>5	2.5	5	2.5	5	2.5	5	2.5	>5	5	>5
Maramec	2.5	5	5	5	1.25	1.25	2.5	2.5	5	>5	2.5	5	2.5	2.5	2.5	5	2.5	>5	5	>5
Caddo	5	5	5	5	1.25	1.25	2.5	2.5	5	>5	2.5	5	5	5	5	5	2.5	>5	5	>5
Jackson	5	5	5	5	1.25	1.25	5	5	5	>5	5	>5	5	5	5	>5	5	>5	5	>5
Creek	2.5	5	5	5	1.25	1.25	2.5	2.5	5	>5	2.5	5	2.5	2.5	2.5	5	2.5	>5	5	>5
Cape Fear	2.5	5	5	5	1.25	2.5	2.5	5	5	>5	2.5	>5	5	5	5	>5	2.5	>5	5	>5
Desirable	5	5	5	5	1.25	2.5	5	5	5	5	2.5	>5	5	5	5	>5	5	>5	5	>5
Moreland	5	5	5	5	1.25	1.25	2.5	2.5	5	5	2.5	5	2.5	2.5	2.5	5	2.5	>5	5	>5
Melrose	5	5	5	5	1.25	1.25	5	5	5	5	5	5	5	5	5	5	5	>5	5	>5
Kiowa	5	5	5	5	1.25	2.5	2.5	5	5	5	2.5	5	5	5	2.5	5	5	>5	5	>5
Nacono	2.5	5	5	5	1.25	1.25	2.5	2.5	5	5	2.5	5	5	5	2.5	5	5	>5	5	>5
Oconee	2.5	5	5	5	1.25	1.25	2.5	2.5	5	5	2.5	5	5	5	2.5	5	5	>5	5	>5
P-cou 2	2.5	5	5	5	1.25	1.25	2.5	2.5	5	5	2.5	>5	5	5	2.5	5	5	>5	5	>5
Success	5	5	5	5	1.25	1.25	5	5	5	5	5	>5	5	5	5	5	5	>5	5	>5
Summer	5	5	5	5	1.25	1.25	2.5	5	5	5	2.5	>5	5	5	2.5	5	5	>5	5	>5
Forkert	2.5	5	5	5	1.25	1.25	2.5	2.5	5	5	2.5	>5	5	>5	2.5	5	5	>5	5	>5
Schley	5	5	5	5	1.25	1.25	2.5	5	5	5	5	>5	5	>5	5	5	5	>5	5	>5
Pawnee	5	5	5	5	1.25	2.5	2.5	2.5	5	5	5	>5	5	>5	5	5	5	>5	5	>5

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7 MIC and MBC values reported in the above table are expressed in mg/mL
8 [1] MIC and MBC values of *E. coli* O157:H7 strains H1730, 658, pecan field isolate, and their respective 3-strain mixture.
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14 Table 2. Effect of method of extraction on minimum inhibitory concentration (MIC) & minimum bactericial concentration (MBC) of pecan shell
15 extracts against different foodborne bacterial pathogens
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Pathogen	Aqueous extracts		Ethanolic extracts	
	MIC	MBC	MIC	MBC
L. monocytogenes				
CDC	1.25	1.25-2.5	1.25	1.25-5
Scott-A	2.5-5	2.5-5	1.25-5	2.5-5
V7	2.5-5	5	2.5	2.5-5
101M	5	5	5	5
4 strain mixture	5	≥5	5	5
Salmonella enterica				
1715	2.5-5	≥5	≥5	>5
PT30	2.5-5	2.5- ≥5	≥5	>5
K4693	2.5-5	≥5	2.5- ≥5	≥5
3 strain mixture	2.5-5	>5	≥5	>5
E. coli O157:H7				
H1730	5	≥5	≥5	>5
658	5	≥5	≥5	≥5
Pecan field	5	≥5	≥5	>5
3 strain mixture	5	≥5	≥5	>5

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18   MIC and MBC values reported in the above table are expressed in mg/mL
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31 Table 3. Survival of *Listeria monocytogenes* on catfish fillets and fresh-cut cantaloupes after treatment with pecan shell extracts and storage at 4°C

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Food Matrix	Type of pecan shell extract	Log survival[1] (CFU/cm^2) of *L. monocytogenes* (4-strain) on pecan shell treated food samples when stored at 4°C for up to 5 days			
		Day-0	Day-1	Day-3	Day-5
Catfish fillets	Control	5.5±0.3^{aA}	6.8±0.1^{aB}	5.0±0.1^{aA}	5.6±0.1^{aA}
	Nacono-Aqueous	2.7±0.1^{bA}	2.8±0.2^{bB}	4.1±0.4^{bC}	5.6±0.6^{bC}
	Nacono-Ethanolic	2.7±0.1^{bA}	2.8±0.1^{bB}	4.1±0.1^{bC}	5.6±0.5^{bcC}
	Caddo-Aqueous	2.9±0.1^{bA}	2.8±0.1^{bB}	4.2±0.1^{bC}	5.5±0.4^{bcC}
	Caddo-Ethanolic	2.8±0.3^{bA}	2.9±0.1^{bB}	4.0±0.1^{bC}	5.4±0.3^{cC}
Fresh-cut cantaloupe	Control	4.1±0.1^{aA}	4.4±0.2^{aA}	5.1±0.9^{aA}	5.9±0.6^{aA}
	Nacono-Aqueous	3.8±0.3^{aA}	3.6±0.3^{aA}	5.3±0.7^{aA}	4.8±0.1^{aA}
	Nacono-Ethanolic	3.9±0.2^{aA}	3.3±0.1^{aA}	5.6±0.5^{aA}	5.8±0.1^{aA}
	Caddo-Aqueous	3.9±0.1^{aA}	4.2±0.1^{aA}	5.1±1.0^{aA}	4.8±1.0^{aA}
	Caddo-Ethanolic	3.7±0.2^{aA}	3.7±0.5^{aA}	5.1±1.0^{aA}	5.3±0.5^{aA}

34 [1] Mean counts with same lowercase superscript within the same column and same uppercase superscript within the same row of individual food
35 matrix type are statistically not significantly different ($P<0.05$) from each other.

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Table 4. Effect of pecan shell extract treatment of catfish fillets and fresh-cut cantaloupe during storage at 5°C on color parameters

Color values[1] of catfish fillet

Treatment type/ Storage time	L* (Lightness)				a* (Greenness)				b*(Blueness)			
	Day-0	Day-1	Day-3	Day-5	Day-0	Day-1	Day-3	Day-5	Day-0	Day-1	Day-3	Day-5
Control	65.4±1.3[bc]	64.3±2.1[c]	67.2±1.2[b]	69.9±1.4[a]	-2.4±0.2[a]	-3.8±2.1[a]	-2.8±0.1[a]	-2.9±0.4[a]	-0.3±0.2[a]	-0.5±1.0[a]	-0.9±1.3[a]	-0.5±1.2[a]
CA	62.4±2.2[b]	60.4±1.9[b]	65.3±1.8[a]	66.9±0.7[a]	-1.1±0.6[a]	-1.5±0.9[ab]	-1.8±0.9[b]	-1.8±1.1[b]	-0.1±1.9[a]	-1.2±2.5[b]	-0.6±1.8[ab]	-0.1±2.1[a]
CE	63.2±2.1[a]	64.1±1.3[a]	63.5±0.4[a]	64.2±0.4[a]	-1.8±0.6[a]	-1.9±0.8[a]	-1.7±0.7[a]	-1.5±0.2[a]	-0.4±1.9[a]	-0.4±2.6[a]	-0.3±0.7[a]	0.3±0.9[a]
NA	61.9±1.3[b]	60.2±1.6[c]	64.7±2.0[a]	64.7±2.4[a]	-0.7±1.1[a]	-0.6±0.8[a]	-1.5±0.6[a]	-1.1±0.5[a]	1.3±1.5[a]	1.4±1.0[a]	-0.1±1.1[a]	0.2±1.2[a]
NE	61.5±2.9[c]	66.1±1.5[b]	66.4±3.8[b]	72.0±1.9[a]	-1.8±1.3[a]	-2.3±1.6[a]	-2.8±0.9[a]	-1.6±1.9[a]	-1.0±2.7[a]	-0.8±2.5[a]	-2.4±1.1[a]	-0.8±1.8[a]

Color values[1] of fresh-cut cantaloupe

Treatment type/ Storage time	L* (Lightness)				a* (Redness)				b*(Yellowness)			
	Day-0	Day-1	Day-3	Day-5	Day-0	Day-1	Day-3	Day-5	Day-0	Day-1	Day-3	Day-5
Control	64.8±3.2[a]	66.0±1.7[a]	61.4±2.0[b]	66.4±3.1[a]	11.4±2.0[ab]	11.8±1.3[a]	9.9±1.2[bc]	8.8±2.0[c]	21.4±3.5[ab]	21.8±2.4[b]	18.5±2.1[ac]	16.1±2.9[c]
CA	63.6±0.9[c]	63.8±1.2[bc]	64.5±0.5[b]	67.5±0.3[a]	14.7±0.5[a]	12.2±0.9[b]	11.9±0.8[bc]	11.1±0.3[bc]	21.9±1.2[a]	17.3±1.5[b]	16.7±1.8[b]	15.8±0.3[b]
CE	70.8±1.5[ab]	69.7±1.2[bc]	68.5±2.1[c]	71.5±0.9[a]	13.1±0.9[a]	12.6±0.7[ab]	12.4±1.5[ab]	11.0±1.1[b]	23.5±0.8[a]	21.6±1.9[ab]	21.6±2.1[ab]	19.2±1.8[b]
NA	65.0±3.9[a]	65.5±1.7[a]	67.2±1.6[a]	66.2±2.5[a]	13.7±3.2[ab]	12.4±1.8[ab]	14.1±1.9[a]	11.8±0.5[b]	20.9±4.3[a]	18.6±2.1[ab]	21.5±2.4[a]	17.3±2.1[b]
NE	67.7±1.2[a]	63.6±1.8[b]	64.9±1.1[b]	64.9±2.3[b]	15.1±1.2[a]	13.3±1.9[ab]	12.7±1.3[bc]	11.2±0.7[c]	24.7±1.4[a]	20.9±3.9[b]	19.4±1.2[bc]	16.8±1.8[c]

[1]Mean color values with same superscript within the same color parameter and the food type are not significantly different (*P>0.05*)

CA- Caddo aqueous extract; CE-Caddo ethanolic extract; NA-Nacono aqueous extract; NE- Nacono ethanolic extract