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2017

Surface Attachment of Active Antimicrobial Coatings Onto Conventional Plastic Based Laminates and Performance Assessment of These Materials on the Storage Life of Vacuum Packaged Beef Sub-Primals

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Clarke, D. et al (2017). Surface attachment of active antimicrobial coatings onto conventional plastic-based laminates and performance assessment of these materials on the storage life of vacuum packaged beef sub-primals. *Food Microbiology*, 62, pp.196-201. doi: 10.1016/j.fm.2016.10.022. E

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Manuscript Details

Manuscript number	FM_2016_189
Title	Surface attachment of active antimicrobial coatings onto conventional plastic-based laminates and performance assessment of these materials on the storage life of vacuum packaged beef sub-primals.
Article type	Research Paper

Abstract

Abstract Two antimicrobial coatings, namely Sodium octanoate and Auranta FV (a commercial antimicrobial composed of bioflavonoids, citric, malic, lactic, and caprylic acids) were used. These two antimicrobials were surface coated onto the inner polyethylene layer of cold plasma treated polyamide films using beef gelatin as a carrier and coating polymer. This packaging material was then used to vacuum pack beef sub-primal cuts and stored at 4°C. A control was prepared using the non-coated commercial laminate and the same vacuum packaged sub-primal beef cuts. During storage, microbial and quality assessments were carried out. Sodium octanoate treated packages significantly ($p < 0.05$) reduced microbial counts for all bacteria tested with an increase of 7 and 14 days, respectively compared to control samples. No significant effect on pH was observed with any treatment. The results suggested that these food grade antimicrobials have the potential to be used in antimicrobial active packaging applications for beef products.

Keywords Gelatin; Coatings; Antimicrobials; Active Food Packaging; Plasma treatment

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1Abstract

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3commercial antimicrobial composed of bioflavonoids, citric, malic, lactic, and
4caprylic acids) were used. These two antimicrobials were surface coated onto the
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13suggested that these food grade antimicrobials have the potential to be used in
14antimicrobial active packaging applications for beef products.

15

161. Introduction

17Microbial contamination has long been recognised as the main source of spoilage
18and reduction of meat quality (Kerry et al., 2012). Prolonging shelf-life, along with
19delivering a high quality food product in suitable flexible packaging, is essential in
20order to address issues such as, global supplier demands, economical profit,
21customer satisfaction, reducing packaging, and more importantly, reduction of food
22wastage. Smart packaging solutions presented in the form of antimicrobially-active
23packaging may be a solution, or part of a solution, to these and other issues (Kerry et
24al., 2008).

25Naturally-occurring active antimicrobial substances offer many potential benefits
26with respect to food packaging applications. Taken from natural food sources,
27potentially edible, and therefore food contact-friendly films and coatings offer new
28alternatives in relation to food packaging materials and packaging applications.
29Edible films and coatings have been used for preservation purposes and can be
30formulated into active materials with the addition of antimicrobial substances (Cuq
31et al., 1995; Campos et al., 2011).

32Bioactive edible/biodegradable films have limitations with respect to certain
33mechanical properties such as transparency, and printability issues. Therefore, such
34limitations mean that these materials will never compete in volume or scale with
35industrially-based conventional packaging materials for some time to come.
36However, the development of such materials should be encouraged as they would
37certainly present novel materials with niche roles and applications, especially when
38used in combination with conventional plastic-based packaging materials

39The use of gelatin to form edible films or coatings has been well documented since
40the 1960's (Hanani et al., 2012a). Films formed using gelatin sources as a primary
41biopolymer packaging film are more desirable to manufactures as they are low cost
42and wide availability (Hanani et al., 2013). Gelatin has been shown to produce films
43with good transparency, mechanical and barrier properties, and can be manufactured
44by extrusion or casting processes (Hanani et al., 2012b, Hanani et al., 2013,
45Molinaro et al., 2015; Wang et al., 2007). There is increased interest in the
46development and use of edible packaging materials to preserve meat quality for
47longer storage periods, while at the same time, maintaining food safety based on
48consumer demands for natural and safe products (Cutter et al., 2006; Ortega et al.,
492014). Polymeric matrices, as well as bioactive coatings and films, can be used as
50carriers for natural antimicrobials. This technique allows for slow diffusion of the
51antimicrobial agent from the packaging material, thus allowing a continuous
52antimicrobial effect on the food product surface over time (Han et al., 2014). This
53approach has been shown to be more effective than spraying, dipping or submerging
54products with or in solutions of antimicrobial agents (Lu et al., 2010, Muriel-Galet et
55al., 2012, Quintavalla & Vincini, 2002, Suppakul et al., 2008 & Yingyuad et al.,
562006). Numerous publications have reported the effects of activating biopolymer-
57based materials with antimicrobial substances for packaging applications (Gómez-
58Estaca et al., 2010, Oussalad et al., 2004, Millette et al., 2007, Gill et al., 2002, &
59Morsy et al., 2014) to name a few. However, limited studies on bovine gelatin as a
60polymer carrier of antimicrobial substances to reduce the microbial growth on fresh
61meat have been reported. Recently our group demonstrated the application of beef
62gelatin films with incorporated antimicrobials as active packaging. Four
63commercially derived antimicrobials namely, Articoat DLP 02 (AR), Artemix Consa
64152/NL (AX), Auranta FV (AFV) (a mixture of citric acid, malic acid, lactic acid and

65caprylic acid in water) and Sodium octanoate (SO) were successively incorporated
66into beef-derived gelatine films and tested for structural, mechanical and barrier
67properties. It was shown that active antimicrobial agents could potentially serve as
68commercial antimicrobial coatings for application onto conventional plastic food
69packaging materials (Clarke et al. 2016).

70To the best of our knowledge, no studies have reported the effect of the use of cold
71plasma treatment to improve attachment properties of activated gelatin solutions
72containing antimicrobials, and the application of the developed coated materials to
73reduce the microbial growth and extend the shelf life of fresh beef sub-primals.
74Therefore, the objective of this study was to examine the effectiveness of surface
75attachment of active beef-derived gelatine antimicrobial (SO and AFV) coatings
76onto conventional commercial vacuum packaging laminates
77(polyethylene/polyamide) and assess the performance of these packaging materials
78on microbial counts of vacuum packaged beef steaks.

792. **Materials and methods**

802.1. **Materials, microbiological media and beef**

81Antimicrobials used in this study included SO, and AFV. SO was obtained from
82Sigma-Aldrich, UK and AFV from Envirotech Innovative Products Ltd, Ireland.
83Glycerol (KB Scientific Ltd, UK) was used as a plasticizer and Beef gelatin 100
84bloom (Helan ingredients, UK) was used as the basal material for all film matrices.
85Two beef subprimal striploins were purchased from a local beef supplier and were
86delivered at 4°C in vacuum-skin plastic packaging formats and used on the same day
87of delivery. Conventional vacuum polyamide/low density polyethylene (PA/LDPE)
88pouches (200x300 mm, water vapour transmission rate of 2.8 g/m² 24 hr and oxygen
89permeability rate of 50 cm³/m² 24 hr) were supplied by Cryovac (Sealed Air W.R.
90Grace Europe Inc., Lausanne, Switzerland) and used as industry standard materials
91for coating and meat packaging trials throughout these studies.

922.2. **Plasma treatment**

93In order to increase the hydrophilicity of the LDPE inner part of the PA/LDPE
94laminated vacuum pouches, cold plasma treatment was carried out on a Dielectric-

95Barrier Discharge (DBD) plasma system prior to the application of the antimicrobial
96coatings.

97 Briefly, pouches of 200 x 300 mm size were cut to size 170 x 280 mm and the
98surface of the LDPE side of the laminate pouches were plasma treated at
99atmospheric pressure using atmospheric air. A schematic representation of the
100experimental setup is presented in Figure 1. The plasma source consisted of two
101circular aluminium plate electrodes (outer diameter = 158 mm). The top dielectric
102barrier was Perspex dielectric barrier (10 mm thickness) and the bottom dielectric
103barrier was polypropylene sheet (5 mm thickness). When the potential across the gap
104reached the breakdown voltage the dielectric barrier prevented the arc transition and
105also homogenised the micro-discharges. The applied voltage was 80 kV which was
106obtained from a step-up transformer (Phenix Technologies, Inc., USA) using a
107variac. The input of 230V, 50 Hz was given to the primary winding of high voltage
108step-up transformer from the mains supply. At the time of treatment, the atmospheric
109air condition was 45% relative humidity (RH) and 22°C. The samples were plasma
110treated for 60 sec to cover the entire film area, leaving only approximately 5 cm from
111the edge of the film (high voltage electrode was placed 1 cm above the film).

112Plasma treatment was carried out on 60 different film samples. Following treatment,
113plasma treated samples were placed in zip lock plastic bags to protect films from
114antistatic environments and dust particles.

115Static contact angle (Theta Lite Optical Tensiometer, Biolin Scientific, UK) was
116measured before and after plasma treatment by the sessile drop technique. A drop of
117each test liquid (water or ethylene glycol) was uniformly placed on the film surface
118using a needle and the image recorded at 15 frames per sec for 10 sec. Images were
119analyzed using the OneAttension software (v 2.1). All values reported are the mean
120of more than 130 data points carried out in triplicate. Surface-free energy was
121calculated using the Owen-Wendt model, utilising the same software. Water and
122ethylene glycol were used as the test liquids.

123Plasma treated film samples were coated with water-based gelatine coatings
124containing the test antimicrobials. For plasma treated films, films were gelatine
125coated after 30 hr of plasma treatment.

1262.3. Coatings of edible antimicrobial films and packaging of beef

1272.3.1. Preparation of film forming solutions and coatings

128 Gelatine film forming solutions were prepared according to the procedure outlined
129 by Wang et al, (2007), with minor modifications. Briefly, dry beef gelatine was
130 dissolved in distilled water (5% w/w) by heating at 90° C in a shaking water bath
131 (SW23, Julabo USA INC., Allentown, PA, USA) for 30 min and glycerol was added
132 under constant stirring at a concentration of 33% w/w dry matter. Successively, the
133 solution was cooled down at room temperature to 40° C before the addition of the
134 antimicrobials. The antimicrobials were added at twice the minimum inhibitory
135 concentration (MIC) determined for each of the two antimicrobials used (Clarke et
136 al., 2016). The MIC of AFV and SO was previously determined at levels of 1.5 and
137 1%, respectively and added at a weight of 3g and 2g respectively (Clarke et al.,
138 2016). The solution was then stirred for 15 min using a sterile magnetic stirrer before
139 casting onto levelled plasma-treated LDPE film surfaces using a Micron II film
140 applicator (Gardco, FL, USA) and dried for 48 hr at 20°C. Gelatine coating thickness
141 was measured using a digital micrometer - Käfer Digital Thickness gauge (Käfer
142 Messuhrenfabrik GmbH & Co., Villingen-Schwenningen, Germany) at 5 random
143 locations of 10 random films for each sample treatment. The thickness of gelatine
144 coating layer ranged between 11–15 µm.

1452.3.2. Vacuum packaging of beef sub-primals using active antimicrobial coated 146 laminate pouches

147 Plasma-treated conventional PA/LDPE films coated with active gelatin-based
148 coatings were detached from the flat surface on which they were coated, the edges of
149 each laminate sample was cleaned with ethanol to remove unwanted residue. Each
150 pack was heat-sealed to form a pouch (170x290 mm) using Webomatic type D463
151 (Webomatic Vacuum Packaging Systems, Werner Bonk, Mausegat 59, D 463
152 Bochum 6, Germany) with the sealing time set at 2.5 s. In order to avoid adhesion
153 between the coated films, sterile food grade aluminium foil was placed between the
154 films prior to sealing.

155 Beef striploins were cut into 90 steaks (2.5 cm in thickness). A total of 30 steaks
156 were individually vacuum packaged in AFV coated pouches, SO coated pouches or

157non-coated pouches without plasma-treatment (control group), respectively using a
158Webomatic type D463 (Webomatic Vacuum Packaging Systems, Werner Bonk,
159Mausegat 59, D 463 Bochum 6, Germany) machine (20 sec vacuum process, 2.5 sec
160heat-seal dwell-time). Vacuum packaged samples were stored under chilled
161conditions of 4°C in a walk-in chilled room and sampled at weekly intervals for up to
1625 weeks.

1632.4. pH

164The pH of the beef steaks was measured using a digital pH metre (Mettler-Toledo
165GmbH, Schwerzenbach, Switzerland) by inserting the glass probe directly into the
166beef steak. Each value represents the average of three readings.

1672.5. Microbiological studies

168Microbiological analyses of beef steaks during storage at 4 °C were performed in
169triplicate on each measurement day. In order to obtain a representative sample, three
170steaks from each treatment were randomly selected for analysis. A total of 10g from
171both the upper and bottom parts of the surface of the steak was aseptically taken
172using sterile forceps and scalpel, placed into a sterile stomacher bag (Seward, UK) to
173which 90 ml of sterile Maximum recovery diluent (MRD) (Oxoid, UK) was added as
174an isotonic diluent and thoroughly mixed for 3 min using a stomacher (Seward, UK)
175in order to obtain a primary 10-fold dilution. This was then 10-fold serially diluted
176using MRD and used to enumerate total viable counts (TVC), psychrotrophic
177bacteria, total anaerobic bacteria (TAB), Lactic acid bacteria (LAB), total coliforms
178and *Escherichia coli* (*E. coli*).

179The TVC and psychrotrophic bacteria were enumerated in Plate count agar (PCA)
180plates (Oxoid, Basingstoke, UK) after incubation for 48 hr at 30 °C or 7 days at 4° C,
181respectively. Total coliforms and *E. coli* were enumerated on Compact Dry E.C
182plates (Nissui Pharmaceutical, Co. Ltd. Japan) (20 cm²) after incubation at 37°C for
18324 hr. LAB were enumerated on overlaid MRSA agar (Oxoid) after incubation for 3
184days anaerobically at 30 °C in anaerobic jars containing Anaerocult® (Merk). TAB
185were enumerated on Tryptone soy agar (TSA) (Oxoid) agar enriched with 0.6% yeast
186extract Oxoid (Basingstoke, UK) after 3 days incubation anaerobically at 30°C in
187anaerobic jars containing Anaerocult®. *Clostridium* spp. were enumerated on

188reinforced *Clostridium* agar (Oxoid) after incubation for 3 days anaerobically at 30°C
189in anaerobic jars containing Anaerocult®. Bacterial numbers were converted to
190log₁₀ colony-forming units per gram sample (CFU/g sample) prior to statistical
191analyses.

1922.6. Statistical analysis

193One-way analysis of variance of data was carried out using the SPSS 22 for
194Windows (SPSS Statistical software, Inc., Chicago, IL, USA) software package.
195Tukey's HSD test was used to compare treatment means when significant differences
196were found with the ANOVA. The significance level was always set to 0.05.

1973. Results and discussions

1983.1. Surface treatment of PE/PA packs

199In a previous study Clarke et al. (2016) demonstrated that SO and AFV displayed the
200greatest antimicrobial efficacy against the tested bacterial strains; (*Bacillus cereus*,
201*Pseudomonas fluorescens*, *Escherichia coli*, *Staphylococcus aureus* and aerobic and
202anaerobic beef microflora) therefore, those antimicrobials were chosen for this study.
203SO and AFV were incorporated in beef gelatine solutions and coated onto the inner
204LDPE of the conventional vacuum PA/LDPE pouches

205

206Beef gelatin hydrophilic water solutions cannot be attached properly onto
207hydrophobic polyethylene surfaces. Polyethylene is by nature a hydrophobic
208material; therefore, it is necessary to modify the surface of the film prior to coating
209to improve wettability and achieve good adhesion (Robertson et al., 2014). There are
210several types of surface modification techniques used in the packaging industry for
211adhesion improvement of coatings, among others; corona treatment, chemical
212treatment or priming (Robertson., 2014) and plasma treatment. Cold plasma induces
213several chemical and physical processes within the plasma volume and on the
214plasma-polymer interface, which modify surface properties (Pankaj et al., 2014).
215Therefore DBD plasma treatment of the LDPE surface was applied in order to
216increase the affinity with the gelatine solution. In order to investigate the surface
217energy properties of the PE before and after plasma treatment the contact angle was

218measured (Yuan and Lee, 2013). The contact angle is defined as the angle Θ formed
219by the intersection of the liquid interface and the surface interface (fig. 2).

220A significant ($p < 0.05$) decrease in the contact angle for both test liquids (water and
221ethylene glycol) was observed after plasma treatment of the surface of LDPE (Table
2221A). The untreated LDPE film had a water contact angle Θ of 97.7° , thereby
223showing the hydrophobic nature of the film. After plasma treatment at 80 kV for 60s
224the water contact angle decreased to 59.9° , thus demonstrating an increase in the
225surface hydrophilicity. Contact angle was also measured during film storage to
226identify if the hydrophobic nature of the film was recovered. A significant ($P < 0.05$)
227increase of the water contact angle was noticed during film storage, reaching a
228contact angle of 73.6° after 75 hr storage at room temperature. The total surface free
229energy (γ^{tot}) increased significantly ($P < 0.05$) due to a significant increase in the polar
230component (γ^p) and consequently, a decrease in the dispersive component (γ^d) (Table
2311B). During film storage, an increase in the dispersive component and decrease in
232the polar component was also observed.

233

234Table 1: Contact angle (A) and surface free energy of DBD plasma treated PE film during
235storage

236

A. Contact angle				
Sample	Storage time (hr)	Water ($^\circ$)	Ethylene glycol ($^\circ$)	
Control (LDPE)	0	97.7 ± 1.5	78.2 ± 1.7	
80kV-60s	0	59.9 ± 3.0	50.9 ± 2.7	
80kV-60s	30	66.9 ± 1.4	37.3 ± 0.4	
80kV-60s	53	66.6 ± 0.7	44.1 ± 3.6	
80kV-60s	75	73.6 ± 0.2	55.2 ± 0.1	

237

B. Surface free energy				
Sample	Storage time (hr)	γ^{tot} [mN/m]	γ^d [mN/m]	γ^p [mN/m]
Control (LDPE)	0	18.2	14.5	3.7
80kV-60s	0	43.2	5.2	38.0
80kV-60s	30	38.7	23.0	15.7
80kV-60s	53	36.4	16.2	20.2
80kV-60s	75	30.5	13.1	17.4

238

239The results presented in this study clearly demonstrate the importance of DBD
240plasma treatment of PE films before coating for making a more homogenous
241antimicrobial coating on the surface of film. However, hydrophobic nature recovery

241of the DBD plasma treated film during storage indicated that the coating process
242must be carried out soon after DBD plasma treatment. In the present study, for
243optimum adhesion and to obtain a more homogeneous film coating LDPE films were
244coated with the antimicrobial coating within 24 hr of plasma treatment.

2453.2. pH values of packaged beef steaks

246The effects of active antimicrobial treatments on the pH values of vacuum packaged
247beef steaks are shown in Table 2. The initial pH of fresh beef in the control packs
248was 5.5. Following the immediate vacuum packaging of steaks using both AFV and
249SO antimicrobial films, steak pH values significantly ($p < 0.05$) increased within 24
250hrs of packaging. This increase in pH was most likely due to the initial pH of the
251antimicrobial coating film forming solutions which were 6.2 and 6.5, respectively. A
252similar effect was reported by Emirođlu et al. (2010) who applied soy protein-based
253edible films (pH 10.0) to beef patty surfaces.

254However, in our study, the increased meat pH was only short-lived, possibly due to
255the initial pH of the beef having a neutralising effect on the overall pH, thus the pH
256decreased quickly and normalised meat pH values were maintained throughout the
257storage time. The decrease in steak pH in antimicrobially-treated packs could be
258attributed to the presence of lactic acid bacteria (LAB). Strains of LAB can produce
259fermentation products in the form of organic acids which may result in a decrease in
260pH over time (Jones, 2004; Sakala et al., 2002). LAB strains of bacteria are known
261to be resistant to many antimicrobial substances (Han et al., 2014; Fik &
262Leszczyska-Fik, 2007; Emirođlu et al. 2010). In our study, we observed the
263successful growth of LAB in our antimicrobially-treated packs (see next section for
264greater detail).

265The opposite trend was observed for the control samples, where a slight increase in
266pH was observed until day 23 of storage. An increase in pH may have been caused
267by the high levels of psychrotrophic bacteria present during the initial days of
268storage. Psychrotrophic bacteria produce volatile amine and ammonia which can
269increase pH levels and that the accumulation of those metabolites produced by
270psychrotrophic bacteria growth may cause pH increase (Cortez-Vega et al., 2012).

272 Table 2. The changes of pH on vacuum packaged beef steaks held in control and antimicrobially-
273 active packaging during chilled storage

Storage days	Control	AFV	SO
1	5.5 ± 0.01 ^a	6.4 ± 0.02 ^b	6.4 ± 0.01 ^b
7	5.5 ± 0.05 ^a	5.6 ± 0.06 ^{ab}	5.7 ± 0.03 ^b
14	5.5 ± 0.04 ^a	5.6 ± 0.02 ^b	5.7 ± 0.01 ^b
23	5.7 ± 0.04 ^a	5.6 ± 0.02 ^{ab}	5.7 ± 0.01 ^b
29	5.6 ± 0.02 ^a	5.6 ± 0.02 ^a	5.7 ± 0.05 ^b
36	5.5 ± 0.02 ^a	5.5 ± 0.02 ^a	5.6 ± 0.01 ^b

274^{ab}Mean values and standard deviations in the same row with different superscripts are significantly
275 different ($p < 0.05$).

276 3.3. Microbiological analysis

277 Initial TVC and psychrotrophic bacteria counts for control, AFV and SO packaged
278 beef steak samples after day 1 of storage were reported as 3.18 log, 2.70 log and 3.00
279 log CFU/g, respectively, indicating that beef steaks were of good microbiological
280 quality (Fig. 3a). In general, during storage independent of the packaging system
281 used, the TVC numbers increased; however, throughout storage higher growth was
282 noticed in control samples compared to samples stored in antimicrobially-coated
283 pouches. Microbiological standards and guidelines give guidance on the types of
284 microorganism and their number that can be considered acceptable or unacceptable
285 or unsafe in a food product. The following recommended microbiological limits are
286 applied for raw meat: Aerobic plate counts: $m = 10^6$ (CFU/ g of meat) (acceptable
287 limit) and $M = 10^7$ (CFU/ g of meat) (Unacceptable limit); *E. coli*: $m = 50$ CFU/g of
288 meat (acceptable limit) and $M = 500$ CFU/ g of meat), (European Commission, 2007;
289 ICMSF, 1986). For this study the limit of acceptability was set to 6 log (CFU/g of
290 meat) and *E. coli* 50 CFU/g of meat. Microbiological enumeration for TVC,
291 psychrotrophic bacteria, LAB, and TAB bacteria during storage are shown in figure
292 3.

293 Throughout storage, significantly ($P < 0.05$) lower bacterial counts were observed in
294 samples that were vacuum packed in packs coated with antimicrobial SO compared
295 to samples that were vacuum packed with control films or films coated with
296 antimicrobial AFV. The results found in this study is in agreement with results
297 reported previously by Clarke et al. (2016) in which SO was the most effective
298 antimicrobial against pure cultures of *B. cereus*, *Ps. fluorescens*, *E. coli*, *S. aureus*

299and the aerobic and anaerobic microflora from beef steaks as it had the lowest MIC
300compared to the other antimicrobials tested. The authors also found that beef-derived
301gelatin films containing SO enhanced the mechanical properties and water vapour
302permeability when compared to control gelatin films.

303The limit of acceptability was reached at day 27, 35 and 42 for samples that were
304vacuum packed with control, AFV coated antimicrobial or SO antimicrobially coated
305films, respectively. Therefore, beef vacuum packed with the antimicrobial coating
306containing AFV or SO stayed within acceptability levels 33 or 55% longer,
307respectively compared to samples that were vacuum packed with control films.

308Significantly ($P<0.05$) slower growth of psychrotrophic bacteria was noticed in beef
309steaks that were vacuum packed with SO coated LDPE films compared to beef
310steaks vacuum packed with AFV coated LDPE films or control films (Fig. 3B).
311However, at the end of storage, no significant differences were noticed between the
312samples that were vacuum packed with AFV or SO coated LDPE films.

313SO films significantly ($p<0.05$) reduced bacterial counts compared to control
314samples for psychrotrophic bacteria from day 14 until the end of testing, AFV treated
315packaging only significantly ($p<0.05$) reduced bacterial counts as compared to
316control packaging on day 21 of testing (Fig. 3B) No significant differences were
317observed at the end of the testing period between AFV and SO films for
318psychrotrophic bacteria.

319A similar pattern was observed on TAB and LAB counts. SO coated LDPE films
320significantly ($p<0.05$) reduced TAB and LAB bacterial counts compared to steaks
321that were vacuum packed with AFV coated LDPE films or control films from day 14
322onwards until the end of testing and storage period (Fig. 3C, 3D). *Clostridium* spp.
323was absent throughout the storage period, coliforms were present on day 35 of
324testing and were slightly higher in treatment samples, however, levels remained
325within acceptable limits (5×10^2) throughout the testing period. Similar results were
326reported in a study conducted by Brightwell et al, (2009) who compared the
327microflora of peroxyacetic acid treated and vacuum-packed beef. Enterobacteriaceae
328counts were first detected on week 6 of testing and were found to be higher in treated
329versus untreated samples, with levels of Enterobacteriaceae being 25% and 11%,
330respectively, of the total microflora present.

331

3324. Conclusion

333 Beef gelatin as a carrier and coating polymer was used to successfully develop
334 antimicrobial gelatine coated LDPE films by coating the inner PE layer of
335 conventional PA/LDPE laminated films after surface activation using cold plasma
336 treatment. The coated antimicrobials tested demonstrated different degrees of
337 effectiveness against bacterial species evaluated on beef steaks. Based on the limit of
338 acceptability in terms of total aerobic counts and *E. coli*, the antimicrobial AFV and
339 SO coated films significantly prolonged the shelf life of beef steaks by 33 or 55%,
340 respectively, compared to samples that were vacuum packed using control film
341 samples. Overall, SO or AFV have the potential of being used as effective
342 antimicrobials for antimicrobial active packaging applications for muscle-based food
343 products.

344

345 Acknowledgement

346 The funding for this research was provided by the Food Institutional Research
347 Measure (FIRM) project entitled 'Packaging and chilling technologies to enhance
348 meat quality and safety' (Pac-Chill-Tech) (11/F/033) under the National
349 Development Plan administered by the Department of Agriculture, Fisheries and
350 Food, Ireland.

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356 References

357Brightwell, G., Clemens, R., Adam, K., Urlich, S., Boerema, J. 2009. Comparison of
358culture-dependent and independent techniques for characterisation of the microflora
359of peroxyacetic acid treated, vacuum-packaged beef. *Food Microbiology*. 3, 383-288.

360Campos, C.A., Gerschenson, L.N., Flores, S.K. 2011. Development of edible films
361and coatings with antimicrobial activity. *Food and Bioprocess Technology*. 4, 849-
362875.

363Clarke, D., Molinaro S., Tyuftin A., Bolton D., Fanning S., Kerry J.P. 2016.
364Incorporation of commercially-derived antimicrobials into gelatin-based films and
365assessment of their antimicrobial activity and impact on physical film properties.
366*Food Control*, 64, 202 – 211.

367Cortez-Vega, W.R., Pizato, S., Prentice, C. 2012. Quality of raw chicken breast
368stored at 5 °C and packaged under different modified atmospheres. *Journal of Food*
369*Safety*. 32, 360–368.

370Cuq, B., Gontard, N., Guilbert, S. 1995. Edible films and coatings as active layers,
371in: Cuq, B., Contard, N., Guilbert, S. (Eds.), *Active food packaging*. Springer, 111-
372142.

373Cutter, C.N. 2006. Opportunities for bio-based packaging technologies to improve
374the quality and safety of fresh and further processed muscle foods. *Meat science*. 74,
375131-142.

376EC (2007). Commission Regulation No 1441/2007 of 5 December 2007 amending
377Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. *Official*
378*Journal of the European Union*. L322, 12-29.

379Emiroğlu, Z.K., Yemiş, G.P., Coşkun, B.K., Candoğan, K. 2010. Antimicrobial
380activity of soy edible films incorporated with thyme and oregano essential oils on
381fresh ground beef patties. *Meat Science*. 86, 283–288.

382Fik, M, Leszczyska-Fik A. 2007. Microbiological and sensory changes in minced
383beef treated with potassium lactate and sodium diacetate during refrigerated storage.
384*International Journal of Food Properties*, 10 (3), pp. 589–598.

385Gómez-Estaca, J., de Lacey, A.L., Gomez-Guillen, M.E., Montero, P. 2010.
386Biodegradable gelatin–chitosan films incorporated with essential oils as
387antimicrobial agents for fish preservation. *Food Microbiology*. 27, 889-896.

388Gill, A.O., Delaquis, P., Russo, P., Holley, R.A. 2002. Evaluation of antilisterial
389action of cilantro oil on vacuum packed ham. *International Journal of Food*
390*Microbiology*. 73, 83-92.

391Han C., Wang. J, Li. Y.,Lu. Cui Y. 2014. Antimicrobial-coated polypropylene films
392with polyvinyl alcohol in packaging of fresh beef. *Meat Science* 96 issue 2, pp 901-
393907.

394Hanani, N.Z.A., Roos, Y.H., Kerry, J.P. 2012. Use of beef, pork and fish gelatin
395sources in the manufacture of films and assessment of their composition and
396mechanical properties. *Food Hydrocolloids*. 29, 144-151.

397Hanani, N.Z.A., Beatty, E., Roos, Y.H., Morris, M.A., Kerry, J.P. 2012. Manufacture
398and characterization of gelatin films derived from beef, pork, and fish sources using
399twin screw extrusion. *Journal of Food Engineering*. 113, 606–614.

400Hanani, N.Z.A., McNamara, J., Roos, Y.H., Kerry, J.P. 2013. Effect of plasticizer
401content on the functional properties of extruded gelatin-based composite films. *Food*
402*Hydrocolloids*. 31, 264–269.

403Jones, R.J. 2004. Observations on the succession dynamics of lactic acid bacteria
404populations in chill-stored vacuum-packaged beef. *International Journal of Food*
405*Microbiology*. 90, 273–282.

406Kerry, J., Paul, B., 2008. Smart Packaging Technologies for Fast Moving Consumer
407Goods, in: Kerry, J.P. (Eds.), *Front Matter*. John Wiley & Sons, Ltd. pp. 1-26.

408Kerry, J.P. 2012. Advances in meat, poultry and seafood packaging, in: Kerry, J.P.
409(Eds.), *A volume in Woodhead Publishing Series in Food Science, Technology and*
410*Nutrition*. Elsevier, pp. 720.

411Millette, M., Le, T.C., Smoragiewicz, W. 2007. Inhibition of *Staphylococcus aureus*
412on beef by nisin-containing modified alginate films and beads. *Food Control*. 18,
413878-884.

414Morsy, M.K., Khalaf, H.H., Sharoba, A.M., El-Tanahi, H.H. 2014. Incorporation of
415Essential oils and Nanoparticles in Pullulan Films to Control Foodborne Pathogens
416on Meat and Poultry Products. *Journal of food science*. 79, 675-684.

417Molinaro, S., Cruz-Romero, M., Sensidoni, A., Morris., M., Lagazio, C., Kerry, J.P.
4182015. Combination of high-pressure treatment, mild heating and holding time effects
419as a means of improving the barrier properties of gelatin-based packaging films
420using response surface modelling. *Innovative Food Science & Emerging*
421*Technologies*. 30, 15-23.

422Muriel-Galet, V., Cerisuelo, J.P., López-Carballo, G., Lara, M., Gavara, R.,
423Hernández-Muñoz, P. 2012. Development of antimicrobial films for microbiological
424control of packed salad. *International Journal of Food Microbiology*. 157, 195–201.

425Pankaj, S.K., Bueno-Ferrer, C., Misra, N.N. 2014. Applications of cold plasma
426technology in food packaging. *Trends in Food Science & Technology*. 35, 5-17.

427Quintavalla, S., Vincini, L. 2002. Antimicrobial food packaging in meat industry.
428*Meat Science*. 62, 373–380.

429Robertson, G.J., 2014. Food packaging: Encyclopedia of Agriculture and Food
430Systems, 2014, pp 232-249

431Sakala. R.M., Hayashidani. H, Kato. Y, Hirata. T, Makino Y, Fukushima A, 2002
432Change in the composition of the microflora, on vacuum-packed beef during chiller
433storage *International Journal of Food Microbiology*, 74 , pp. 87–99

434Suppakul, P., Sonneveld, K., Bigger, S.W., Miltz, J. 2008. Efficacy of polyethylene-
435based antimicrobial films containing principal constituents of basil. *LWT-Food*
436*Science and Technology*. 41, 779–788.

437Wang, L., Liu, L., Holmes, J., Kerry, J.F., Kerry, J.P. 2007. Assessment of film-
438forming potential and properties of protein and polysaccharide-based biopolymer
439films. *International Journal of Food Science and Technology*. 42, 1128–1138.

440Yingyuad, S., Ruamsin, S., Reekprkhon, D., Douglas, S., Pongamphai, S.,
441Siripatrawan, U. 2006. Effect of chitosan coating and vacuum packaging on the
442quality of refrigerated grilled pork. *Packaging Technology and Science*. 19, 149–157.

443 Yuan, Y, Lee T. R. Contact Angle and Wetting Properties. Chapter in Surface Science
444 Techniques. Springer Series in Surface Sciences. 51, 3-34.

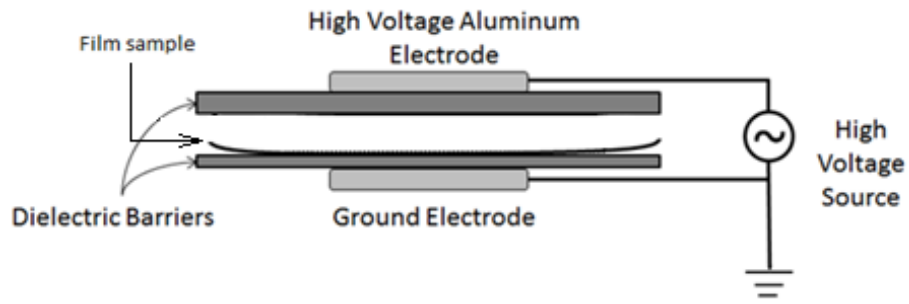


Fig. 1. Schematic representation of the experimental setup for plasma treatment.

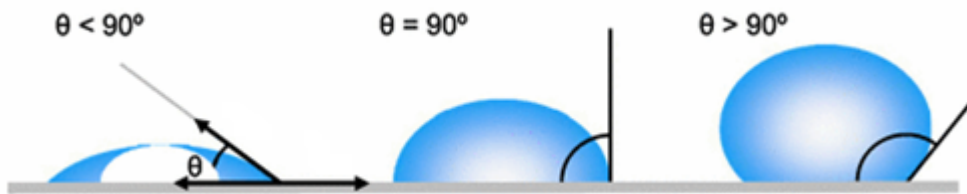
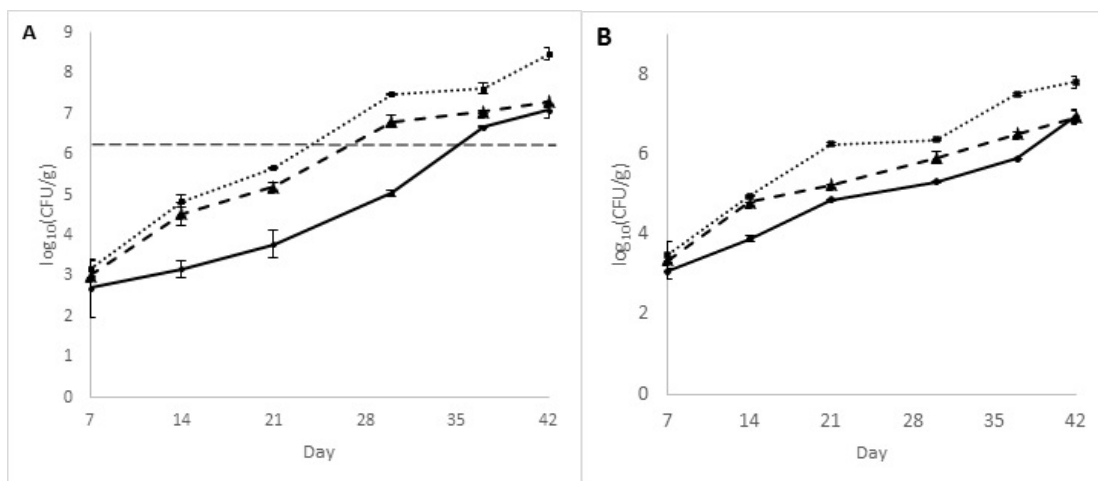


Fig. 2. Contact angle formed by sessile liquid drops on a smooth homogeneous solid surface (hydrophilic surface ($\theta < 90^\circ$) hydrophobic surface ($\theta > 90^\circ$), (Yuan and Lee, 2013).



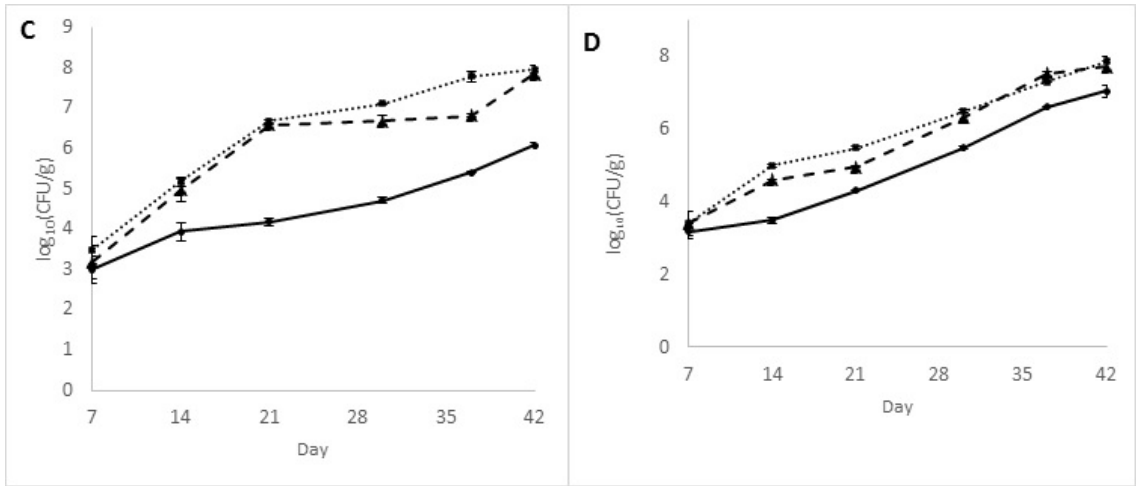


Fig. 3. Changes in (A) Total viable counts, (B) Psychrotrophic bacteria (C) Lactic acid bacteria, and (D) Total anaerobic bacteria during chilled storage of beef steaks vacuum-packaged using control (...) or antimicrobially-active (---) AFV or (—) SO packaging films. The dotted line in (A) indicates the limit of acceptability. Bars represent standard deviation of three independent samples and may be hidden by symbols when small.

- Beef gelatin was successfully coated on plasma treated polyethylene surface
- Auranta FV and Sodium octanoate coated films prolonged the shelf life of beef cuts
- AFV and SO potentially can be used in antimicrobial active packaging

Surface attachment of active antimicrobial coatings onto conventional plastic-based laminates and performance assessment of these materials on the storage life of vacuum packaged beef sub-primals.

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KEYWORDS: Gelatin films; Edible coatings; Antimicrobials; Antimicrobial Active Packaging; Food Packaging; Plasma treatment