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### 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  
5inner polyethylene layer of cold plasma treated polyamide films using beef gelatin as  
6a carrier and coating polymer. This packaging material was then used to vacuum  
7pack beef sub-primal cuts and stored at 4°C. A control was prepared using the non-  
8coated commercial laminate and the same vacuum packaged sub-primal beef cuts.  
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10octanoate treated packages significantly ( $p < 0.05$ ) reduced microbial counts for all  
11bacteria tested with an increase of 7 and 14 days, respectively compared to control  
12samples. No significant effect on pH was observed with any treatment. The results  
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/m<sup>2</sup> 24 hr and oxygen  
89permeability rate of 50 cm<sup>3</sup>/m<sup>2</sup> 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 cm<sup>2</sup>) 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  
190log<sub>10</sub> 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  $\Theta$  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  $\Theta$  of  $97.7^\circ$ , thereby  
 223showing the hydrophobic nature of the film. After plasma treatment at 80 kV for 60s  
 224the water contact angle decreased to  $59.9^\circ$ , 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  $73.6^\circ$  after 75 hr storage at room temperature. The total surface free  
 229energy ( $\gamma^{tot}$ ) increased significantly ( $P<0.05$ ) due to a significant increase in the polar  
 230component ( $\gamma^p$ ) and consequently, a decrease in the dispersive component ( $\gamma^d$ ) (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	$97.7\pm 1.5$	$78.2\pm 1.7$	
80kV-60s	0	$59.9\pm 3.0$	$50.9\pm 2.7$	
80kV-60s	30	$66.9\pm 1.4$	$37.3\pm 0.4$	
80kV-60s	53	$66.6\pm 0.7$	$44.1\pm 3.6$	
80kV-60s	75	$73.6\pm 0.2$	$55.2\pm 0.1$	

237

B. Surface free energy				
Sample	Storage time (hr)	$\gamma^{tot}$ [mN/m]	$\gamma^d$ [mN/m]	$\gamma^p$ [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 <sup>a</sup>	6.4 ± 0.02 <sup>b</sup>	6.4 ± 0.01 <sup>b</sup>
7	5.5 ± 0.05 <sup>a</sup>	5.6 ± 0.06 <sup>ab</sup>	5.7 ± 0.03 <sup>b</sup>
14	5.5 ± 0.04 <sup>a</sup>	5.6 ± 0.02 <sup>b</sup>	5.7 ± 0.01 <sup>b</sup>
23	5.7 ± 0.04 <sup>a</sup>	5.6 ± 0.02 <sup>ab</sup>	5.7 ± 0.01 <sup>b</sup>
29	5.6 ± 0.02 <sup>a</sup>	5.6 ± 0.02 <sup>a</sup>	5.7 ± 0.05 <sup>b</sup>
36	5.5 ± 0.02 <sup>a</sup>	5.5 ± 0.02 <sup>a</sup>	5.6 ± 0.01 <sup>b</sup>

274<sup>ab</sup>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 ( $5 \times 10^2$ ) 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

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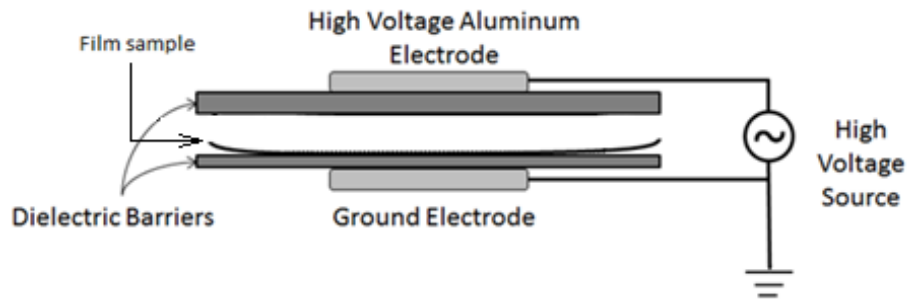
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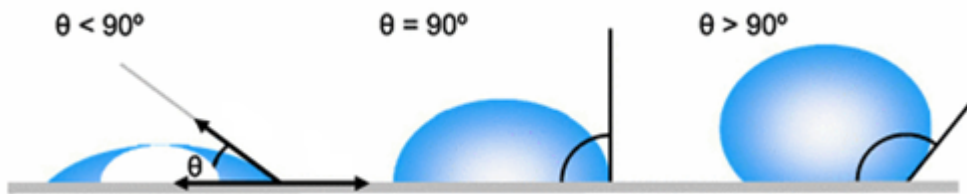
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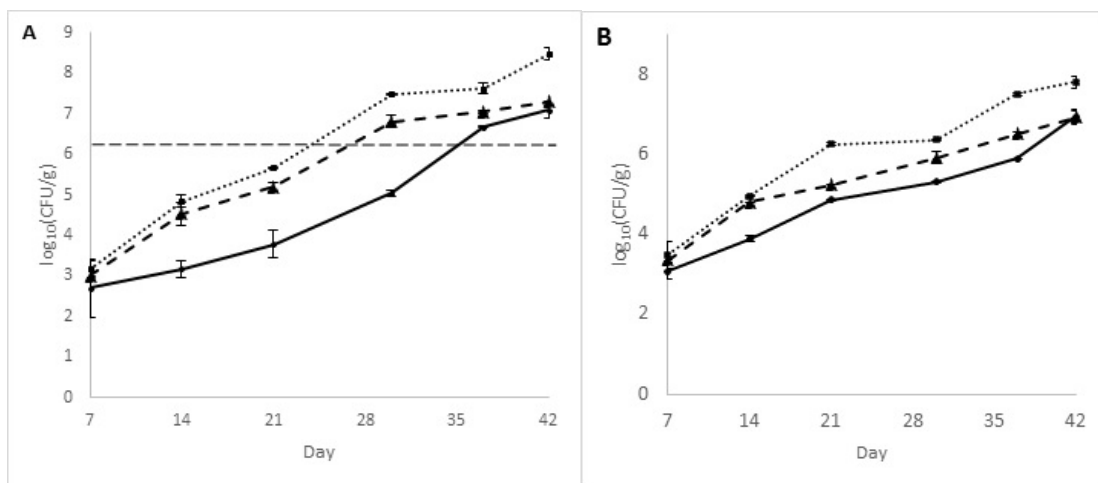
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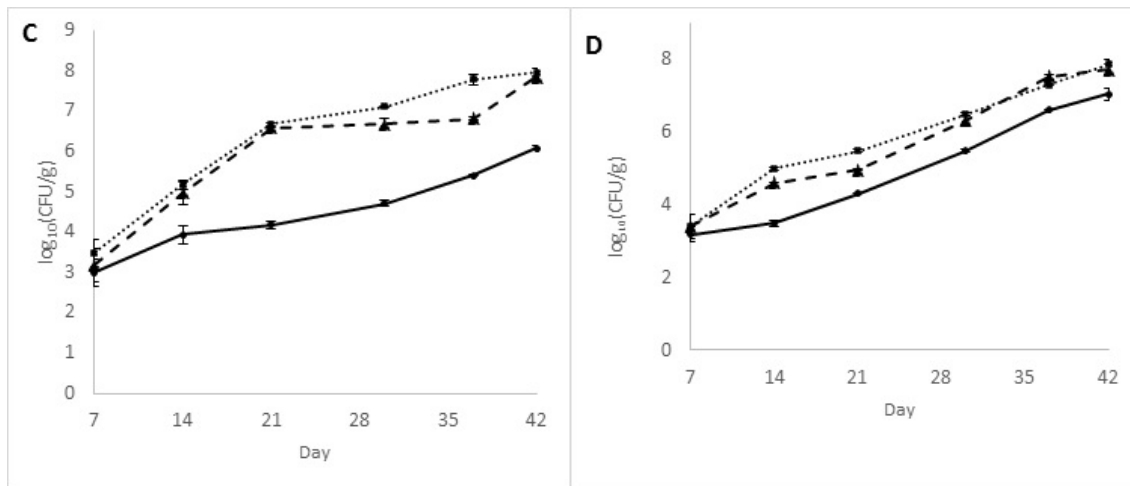


**Fig. 1.** Schematic representation of the experimental setup for plasma treatment.



**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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