Inhibition of foodborne bacteria by antibacterial coatings printed onto food packaging films

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Abstract Films containing antibacterial compounds could be used for packaging perishable foods such as fresh fish and meat for sea freighting over long distances. However, existing commercialised options (films with nanosilver zeolites or wasabi extract) are only permitted for food contact in certain regions and films containing alternative antibacterial ingredients are required e.g. for exports to Europe. Certain non-volatile phenolic plant extracts have shown promising antibacterial activity against a wide range of foodborne bacteria in in vitro assays and when integrated in coatings for perishable foods such as fish and meat. Extracts rich in gallotannins tend to show stronger antibacterial effects than other phenols such as flavonoids. Such extracts could be coated onto commercial barrier films by means of flexographic printing—a more industrially feasible option than rod coating or solvent casting typically used in antibacterial coating research. The goal of the present work was to investigate the antibacterial effect of printed latex coatings containing extracts rich in gallotannins and other types of phenolic compounds against 16 common spoilage and pathogenic bacteria of fish and meat. The largest zones of inhibition in disk diffusion assays were obtained with plastic films with coatings containing tannic acid alone, followed by tannic acid with phenolic-rich extracts of feijoa skin or mango seed. Significant inhibition was seen for all bacteria. This study shows that coatings with gallotannins as the main active ingredient can be printed onto commercial barrier films to

control the bacteria that limit the shelf-life of fresh fish and meat.

Keywords Coatings · Bacteria · Inhibition · Shelf-life · Printing

Introduction

Chilled fish and meat vacuum-packaging for export usually uses multilayer barrier films. Because of the relatively short shelf-life of chilled fish, sea freighting from New Zealand is not possible and air-freighting is the only option. One approach to extend shelf-life by controlling spoilage bacteria (Shaw and Shewan 1968; Gram and Dalgaard 2002; Adam et al. 2010) and reduce the risk from pathogenic bacteria (that might be introduced to food surfaces during processing or handling) would be to coat the packaging film with a carrier polymer containing antibacterial agents (Suppakul et al. 2003; Joerger 2007; Kirwan et al. 2011). Antimicrobial agents can also be embedded in the packaging film itself or applied in an edible coating deposited onto the food or the packaging film surface (Suppakul et al. 2003; Joerger 2007). These applications are mostly based on migration of volatile or non-volatile antibacterial agents from the matrix to the food surface to inhibit bacterial growth. There is one commercial application based on this concept: films containing wasabi extract (Lee et al. 2015). For example, WasaOuro (Mitsubishi-Kagaku Foods Co., Japan) comprises a plastic film coated with cyclodextrin-encapsulated wasabi extract, emitting the volatile antimicrobial compound allyl isothiocyanate. There are also commercial packaging films containing nanosilver zeolites (Silvestre et al. 2011). However, the food packaging legislation of many regions, including Europe, does not allow

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the use of allyl isothiocyanate or nanosilver films for food contact.

In recent years, a large number of research papers have been published on packaging films/coatings for seafood (Min et al. 2008; Ye et al. 2008; Galotto et al. 2012; Iturriaga et al. 2012; López de Lacey et al. 2014) and meat (La Storia et al. 2013; Badr et al. 2014; Blanco Massani et al. 2014; Han et al. 2014; Higuera et al. 2014). The films and coatings contained antibacterial ingredients such as essential oils or their components (Burt 2004; Kuorwel et al. 2011), non-volatile phenolic compounds (Chung et al. 1993; Balasundram et al. 2006; Peschel et al. 2006; Engels et al. 2009; Ayala-Zavala et al. 2010; López de Lacey et al. 2014; Widsten et al. 2014), bacteriocins (La Storia et al. 2013; Blanco Massani et al. 2014), and enzymes (Min et al. 2008). Despite the large volume of work in this field, there is still no universally approved and commercialised antimicrobial food packaging film on the market. Besides legislative compliance issues, this could be due to inactivation or evaporation of the antimicrobial agents under the harsh conditions (high temperature, pressure and shear forces) that are typically required for commercial film manufacturing methods such as heat extrusion. A further reason might be the negative impact of antimicrobial agents on the sensory properties of food. Many of the above-cited publications used coating or film preparation methods such as solvent casting or rod coating which are not normally available to packaging film manufacturers. While extrusion is only an option for thermally stable active ingredients of any type, non-volatile antibacterial compounds (including heat-labile compounds) could be incorporated onto commercial food packaging films by the flexographic printing used by manufacturers to print text and images onto their films. The printers have in-line corona treaters for activating the film surface for coating and up to eight printing units (ink stations) with drying stations. This setup allows one to deposit multiple superimposed coatings onto the food contact surface (often polyethylene) of packaging films. Exposure of sensitive ingredients to high temperatures can be avoided as long as the drying temperatures are kept sufficiently low. Because of these advantages and its availability to film manufacturers, we decided to use flexographic printing to apply antibacterial coating formulations onto packaging film.

A previous study had been conducted to screen a large number of tree- and fruit-based extracts against common spoilage bacteria and potential pathogens in fish and meat (Widsten et al. 2014). The three most effective extracts were mango seed extract (MSE), tannic acid (TA) and feijoa skin extract (FSE). The phenolic compounds in TA and MSE were found to be mainly polygalloyl glucoses (gallotannins), while MSE also contained other prominent components such as mangiferin, a xanthone C-glycoside.

Gallotannins (Haddock et al. 1982; Chung et al. 1993; Engels et al. 2009, 2010) and mangiferin (Matkowski et al. 2013) have proven antibacterial properties (Fig. 1). The antibacterial effects of gallotannins are reportedly due to their iron-chelating ability, i.e., they deprive bacteria of the iron they need to grow. FSE was found to be a heterogeneous mixture of many different classes of phenolic compounds, including catechin, gallic acid, ellagitannins and phenolic acids (Fig. 1).

The goals of this study were to (1) evaluate the suitability of flexographic printing for coating commercial packaging films with antimicrobial coatings and (2) investigate the effectiveness of printed coatings containing predominantly phenolic-type plant extracts for inhibition of fish- and meat-borne bacteria.

Materials and methods

Commercial extracts, chemicals and solvents

TA and gallic acid were purchased from Sigma-Aldrich. The acetone used for the extractions was of analytical grade and supplied by Merck.

Extract raw materials and preparation

MSE was prepared from ripe Australian Pearl mangoes (*Mangifera indica* L.) supplied by a New Zealand wholesaler and FSE from ripe feijoas (*Feijoa sellowiana*) purchased from a local supermarket. The seeds were removed from the mangoes manually and the fibrous endocarps surrounding the kernels discarded. Feijoa skins and pulp were segregated manually and the skins rinsed clean of juice residue with deionised water. The mango kernels and feijoa skins were then freeze-dried and ground into powders through a 1 mm mesh using a Wiley mill. MSE and FSE were extracted in batches of 100 g of dry powder in 80% aqueous acetone (or water in the case of MSE only) at a consistency of 10% in 1 L Schott bottles. The bottles were shaken at 150 rpm for 90 min at 50 °C, their contents centrifuged (3000 rpm, 5 min, room temperature) and the supernatants removed by decanting. The acetone was then removed under reduced pressure at 30 °C and the remaining mixture of water and extract freeze-dried to obtain MSE or FSE. The first 100 g mango seed was re-extracted with the same method to find out how completely the phenolic extractives had been removed by the first extractions.

Total phenol content (TPC) determination

The total phenol content of extracts (including TA) was determined in triplicate by the Folin–Ciocalteu method

Fig. 1 Examples of the phenolic compounds found in tannic acid, mango seed extract and feijoa skin extract

(Kähkönen et al. 1999) and expressed as gallic acid equivalents (GAE). Appropriate dilutions were prepared from stock solutions made by dissolving 250 mg of extract in 99.75 g of Milli-Q water. In the case of MSE and FSE, extracts from different batches were analysed separately.

Selection of target bacteria

The bacteria selected for antibacterial testing included spoilage bacteria typically found in chilled fish and/or lamb (*Shewanella putrefaciens*, *Pseudomonas fluorescens*, *Serratia liquefaciens*, *Photobacterium phosphoreum*, *Brochothrix thermosphacta*, *Hafnia alvei*, *Serratia proteomaculans*, *Clostridium estertheticum* and *Lactobacillus* sp.) as well as a number of pathogenic bacteria (*Escherichia coli* 0157:H7, *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Listeria monocytogenes*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* ATCC 25923 and *Yersinia enterocolitica*). The former are relevant for extending food shelf-life while the latter may contaminate food during handling or storage and can cause serious foodborne infections. Most bacterial strains were sourced from The NZ Institute

for Plant and Food Research Limited culture collection, while *E. coli*, MRSA and *B. thermosphacta* were acquired from the Environmental Science and Research Centre, and *C. estertheticum* and *S. proteomaculans* were kindly supplied by AgResearch, NZ. *Brochothrix*, *Clostridium*, *Lactobacillus*, *Listeria* and *Staphylococcus* are Gram-positive genera while the remainder are Gram-negative.

Preparation of antibacterial coatings

Coating formulations were prepared by dissolving extract powders directly in aqueous carboxylated styrene-butadiene latex (DTO 6100 from Styron, Jakarta, Indonesia) to give an extract-to-latex solids weight ratio of 1:4. Equal proportions of each extract were used for formulations containing mixed extracts. The latex had a solids content of 53%, particle size of 140 nm and pH of 6. The mixtures were homogenised by stirring them mechanically at 1000 rpm for at least 20 min and diluting with deionised water where necessary to adjust the viscosity to 300–750 cP (measured with a Brookfield DV-II+ Pro viscometer and its spindle S63 at a speed of 100 rpm). The coated substrate was a commercial multilayer food packaging film (Cryovac® BLUS 50) supplied by SealedAir,

Hamilton, NZ. Pieces of film measuring 25 cm × 25 cm were cut off and secured onto linerboard with adhesive tape around the edges. The food contact side of the film (polyethylene) was then wiped clean with analytical grade 96% ethanol and corona-treated with a bench-scale device shortly before applying the coating. A flexographic printer (RK Print K303 Multi Coater) operating at a speed of 30 m/min was used to coat the formulations onto the corona-treated film surfaces. A number of super-imposed coats were printed on each film, depending on the viscosity of the coating solution, allowing each coat to air dry for at least 10 min at room temperature before the next one was printed. After the final coat had dried, the untreated edges of the film were cut off and discarded. Coating thickness was determined by deducting the thickness of the uncoated film from that of the coated films. The thickness measurements were carried out with a Lortzen & Wettre micrometer. Two coated films were produced from each of seven coating formulations (TA, MSE, FSE, TA-MSE, TA-FSE, MSE-FSE, TA-MSE-FSE) with low and high coating thicknesses of 75–78 nm (4–8 coats) and 80–85 nm (5–12 coats), respectively (Fig. 2).

Antibacterial testing

Preparation of bacterial strains

The selected bacterial strains were recovered in Brain Heart Infusion (BHI, Difco) and kept on blood agar plates

(Fort Richard, NZ) at 4 °C for storage until the experimental day. Bacteria were cultured in Mueller–Hinton broth (MHB, Difco) except for *L. monocytogenes* (grown in Trypticase Soya Broth supplemented with 0.6% of Yeast Extract, TSBYE, Difco) for 24–48 h prior to the experiments. Each culture was incubated at its optimal growth temperature to achieve 1×10^8 to 1×10^9 colony-forming units/ml (CFU/mL).

Disk diffusion assays

Coated films were aseptically cut using a 6 mm core borer. The disks were marked to identify the active face of the film. The disk diffusion assay was performed according to NCCLS (1993) with some minor modifications. Bacterial cultures were centrifuged for 5 min at 4000 rpm, washed twice with phosphate buffered saline solution and resuspended in 10 mL of 1% Salt Peptone Water (SPW: 1% NaCl, 0.1% Difco peptone) to achieve a final concentration of 10^5 – 10^6 CFU/mL. An aliquot (100 µL) of each bacterial suspension was placed into a Petri dish containing 20 mL of solidified Mueller–Hinton agar (MHA) or Trypticase Soya Agar supplemented with 0.6% Yeast Extract (TSAYE, Difco) for *L. monocytogenes*, and spread using a disposable sterile spreader. The plates were left to dry in the laminar flow for ca. 30 min. Using a sterile stainless steel forceps, individual 6 mm disks were carefully placed onto the agar applying the active face of film towards the

Fig. 2 Application of antibacterial coatings on packaging film (=substrate)

agar. In each Petri dish a maximum of 12 films were tested. Each of the bacteria was then incubated at its optimum growth temperature (from 20 to 37 °C) for 24–48 h. The zones of (growth) inhibition (ZIs) surrounding the disks were measured with a digital calliper. A control disk (film without coating) was included on each tested plate. Two independent experiments were conducted in triplicate.

Statistical analysis

Each combination was tested twice (Experiments 1 and 2) in triplicate and the results subjected to statistical analysis ($P = 0.05$). As an initial analysis for the role of bacteria and compounds in their inhibition, the mean ZI for each combination in each Experiment was analysed using two-factor ANOVA (Bacteria, Compound and their interaction). Experiment 1 was included as a blocking factor since the ZIs tended to be higher in Experiment 2. A regression model was then applied to look at the effects of individual compounds in the combinations: one column for each of TA, MSE, FSE, TA High, MSE High and FSE High, with a 1 where the compound is present and 0 otherwise. This model meant that we could also look for interaction effects which occur when both compounds are present over and above their individual effects.

Results and discussion

Extract yield and total phenol content

During the extraction of antimicrobial compounds from raw materials, 80% acetone proved to be a better solvent for MSE than water in terms of yield and TPC (Table 1). Re-extracting the same material yielded much less extract while TPC was significantly reduced, indicating that the first extractions removed most of the phenolic material. MSE and FSE coatings were therefore made after one extraction in 80% acetone. The TPC of the Australian Pearl MSE (first extraction with 80% acetone) and FSE were similar to those reported earlier (Widsten et al. 2014) for extracts from Tommy Atkins mango seeds (endocarp + kernel) and for the same batch of feijoa skins that

was used in the present study. The TPC of the TA from Sigma-Aldrich was somewhat lower than that from BDH Laboratory Supplies (885 ± 55 mg GAE/g) tested previously.

Antibacterial effects of coatings

Antibacterial activity of natural compounds against 16 bacteria (foodborne pathogens and spoilage microorganisms) was qualitatively and quantitatively assessed based on the presence of an inhibition zone (ZI) and the measurement of its diameter using the disc diffusion assay (Table 2).

Antibacterial activity of each extract in terms of the ZI obtained increased with an increase in coating thickness (Table 2). On average, TA-Low and TA-High showed the largest antimicrobial activity (ZIs of 7.9 and 8.3 mm, respectively). TA-High was significantly the most effective compound ($p < 0.001$). None of the other compounds tested singly (MSE and FSE) had such an effect with average ZI values varying from 5.4 to 5.5 mm and 4.5 to 4.8 mm, respectively, for ‘low’ and ‘high’ coatings (Table 2).

Many natural compounds such as essential oils are known to show synergistic antimicrobial activity when used in combination (Calo et al. 2015). The effect of combining the compounds was also evaluated in this study. The interaction between TA and/or FSE and MSE did not significantly increase the antimicrobial activity against any of the microorganisms tested ($p < 0.001$). The average ZIs obtained for all the interactions were smaller than the ones presented by TA alone. When the combination comprised only MSE and FSE (‘low’ and ‘high’), the average ZI values were significantly lower ($p > 0.001$) (Table 2).

Looking at the average ZI values of ZI, the most effective combinations were TA-FSE High (8.1 mm), TA-FSE Low (7.9 mm) and TA-MSE High (7.8 mm). It is noteworthy that also those combinations included TA (Table 2). These interactions were not significantly different ($p < 0.001$).

Individually, FSE and MSE had no significant effect on ZI ($p = 0.782, 0.467, 0.124$ and 0.092 for FSE-Low, FSE-High, MSE-Low and MSE-High, respectively). The

Table 1 Extract yield and total phenol content ($\pm$ SE)

Extract	Solvent	No. of batches extracted	Yield (% d. m.)	TPC (mg GAE/g)
FSE	80% acetone	2	25.9 ± 2.9	174 ± 12
MSE, 1st extraction	80% acetone	9	18.4 ± 1.0	419 ± 23
MSE, 2nd extraction	80% acetone	1	4.3	249 ± 17
MSE, 1st extraction	Water	1	15.2	257 ± 2
MSE, 2nd extraction	Water	1	4.7	249 ± 4
TA	n.a.	n.a.	n.a.	727 ± 12

Table 2 Zones of inhibition (mean $\pm$ SD, $n = 6$) in the disk diffusion test for different spoilage and pathogenic bacteria

Test bacterium	TA (Low)	TA (High)	MSE (Low)	MSE (High)	FSE (Low)	FSE (High)	TA-MSE (Low)	TA-MSE (High)	TA-FSE (Low)	TA-FSE (High)	MSE-FSE (Low)	MSE-FSE (High)	TA-MSE-FSE (Low)	TA-MSE-FSE (High)
<i>Shewanella putrefaciens</i>	5.2 $\pm$ 4.4	5.9 $\pm$ 3.5	4.1 $\pm$ 4.5	4.8 $\pm$ 4.4	4.1 $\pm$ 4.6	4.0 $\pm$ 5.1	5.0 $\pm$ 3.7	6.3 $\pm$ 3.8	4.4 $\pm$ 4.9	5.0 $\pm$ 4.6	0.0 $\pm$ 0.0	4.5 $\pm$ 4.9	0.0 $\pm$ 0.0	2.8 $\pm$ 5.1
<i>Photobacterium phosphoreum</i>	10.1 $\pm$ 5.8	11.5 $\pm$ 4.5	6.2 $\pm$ 3.1	4.8 $\pm$ 3.8	6.7 $\pm$ 2.3	7.5 $\pm$ 3.4	9.4 $\pm$ 3.3	9.6 $\pm$ 3.7	10.0 $\pm$ 4.9	7.7 $\pm$ 5.0	1.3 $\pm$ 2.0	2.3 $\pm$ 3.7	5.5 $\pm$ 4.7	8.4 $\pm$ 3.3
<i>Bacillus thermosphacta</i>	7.2 $\pm$ 3.4	8.2 $\pm$ 4.0	6.7 $\pm$ 3.4	6.2 $\pm$ 3.8	5.4 $\pm$ 3.3	6.8 $\pm$ 3.2	8.0 $\pm$ 2.8	7.5 $\pm$ 3.4	7.5 $\pm$ 2.4	7.7 $\pm$ 3.6	5.2 $\pm$ 3.5	6.3 $\pm$ 4.0	7.0 $\pm$ 3.6	7.4 $\pm$ 4.1
<i>Pseudomonas fluorescens</i>	14.7 $\pm$ 4.2	13.2 $\pm$ 4.4	9.0 $\pm$ 4.3	9.6 $\pm$ 5.7	8.1 $\pm$ 4.2	9.3 $\pm$ 4.8	12.0 $\pm$ 5.2	12.3 $\pm$ 4.2	13.5 $\pm$ 3.6	14.6 $\pm$ 4.2	7.9 $\pm$ 4.3	10.1 $\pm$ 4.3	10.6 $\pm$ 4.3	12.5 $\pm$ 5.6
<i>Hafnia alvei</i>	4.8 $\pm$ 5.3	4.4 $\pm$ 4.0	0.4 $\pm$ 0.9	1.3 $\pm$ 3.2	1.4 $\pm$ 3.5	1.4 $\pm$ 3.4	1.0 $\pm$ 1.6	6.0 $\pm$ 2.8	5.4 $\pm$ 5.9	5.3 $\pm$ 4.8	1.3 $\pm$ 3.1	0.0 $\pm$ 0.0	1.6 $\pm$ 3.9	0.0 $\pm$ 0.0
<i>Serratia liquefaciens</i>	7.0 $\pm$ 5.2	6.7 $\pm$ 4.2	4.6 $\pm$ 5.0	5.6 $\pm$ 5.2	1.5 $\pm$ 3.7	0.0 $\pm$ 0.0	5.9 $\pm$ 4.6	6.6 $\pm$ 4.2	6.7 $\pm$ 5.2	6.5 $\pm$ 4.0	0.0 $\pm$ 0.0	1.5 $\pm$ 3.6	3.1 $\pm$ 4.8	5.5 $\pm$ 5.0
<i>Serratia proteomaculans</i>	5.8 $\pm$ 6.1	7.6 $\pm$ 4.3	5.5 $\pm$ 4.7	5.8 $\pm$ 5.2	1.5 $\pm$ 3.8	3.3 $\pm$ 5.2	7.0 $\pm$ 3.9	6.8 $\pm$ 3.8	6.8 $\pm$ 6.3	6.5 $\pm$ 3.9	0.0 $\pm$ 0.0	3.2 $\pm$ 4.9	5.4 $\pm$ 6.0	3.8 $\pm$ 4.1
<i>Clostridium estertheticum</i>	5.5 $\pm$ 3.6	7.3 $\pm$ 2.3	4.5 $\pm$ 5.0	2.9 $\pm$ 4.4	5.4 $\pm$ 4.1	5.9 $\pm$ 2.6	6.8 $\pm$ 2.5	6.7 $\pm$ 2.4	7.7 $\pm$ 2.0	7.7 $\pm$ 2.2	3.9 $\pm$ 4.3	4.1 $\pm$ 4.4	2.6 $\pm$ 4.1	4.9 $\pm$ 4.1
<i>Escherichia coli</i> 0157:H7	8.0 $\pm$ 1.5	8.6 $\pm$ 0.9	6.8 $\pm$ 2.5	6.8 $\pm$ 3.1	5.0 $\pm$ 3.4	3.8 $\pm$ 3.3	7.4 $\pm$ 1.5	6.5 $\pm$ 3.8	8.2 $\pm$ 1.9	8.6 $\pm$ 0.7	2.2 $\pm$ 3.7	6.7 $\pm$ 2.5	4.9 $\pm$ 5.0	5.9 $\pm$ 3.5
<i>Lactobacillus</i> sp.	7.7 $\pm$ 1.1	6.1 $\pm$ 1.8	5.4 $\pm$ 1.2	6.3 $\pm$ 2.2	6.0 $\pm$ 1.4	5.6 $\pm$ 1.3	5.6 $\pm$ 1.9	5.1 $\pm$ 1.6	6.7 $\pm$ 2.6	5.7 $\pm$ 2.3	4.1 $\pm$ 1.3	2.5 $\pm$ 4.3	5.0 $\pm$ 1.6	5.3 $\pm$ 1.8
<i>Salmonella</i> Enteritidis	7.3 $\pm$ 5.1	7.6 $\pm$ 4.7	6.7 $\pm$ 3.5	6.3 $\pm$ 3.9	3.2 $\pm$ 4.9	5.5 $\pm$ 5.2	7.4 $\pm$ 4.1	7.7 $\pm$ 4.3	7.2 $\pm$ 6.0	8.2 $\pm$ 4.8	5.5 $\pm$ 4.1	6.0 $\pm$ 3.8	5.5 $\pm$ 4.2	6.2 $\pm$ 3.9
<i>Salmonella</i> Typhimurium	8.1 $\pm$ 7.0	9.8 $\pm$ 6.7	6.7 $\pm$ 4.9	5.9 $\pm$ 5.1	5.8 $\pm$ 6.3	5.8 $\pm$ 6.4	7.9 $\pm$ 4.6	8.1 $\pm$ 5.2	8.4 $\pm$ 5.9	9.1 $\pm$ 6.4	5.1 $\pm$ 5.6	5.9 $\pm$ 5.5	4.1 $\pm$ 5.8	9.0 $\pm$ 5.6
<i>Listeria monocytogenes</i>	7.9 $\pm$ 2.4	9.2 $\pm$ 0.6	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	3.8 $\pm$ 3.4	8.7 $\pm$ 1.1	8.6 $\pm$ 1.4	9.4 $\pm$ 1.3	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.6 $\pm$ 1.5	4.3 $\pm$ 3.5
<i>Staphylococcus aureus</i> MRSA	8.4 $\pm$ 3.1	8.6 $\pm$ 3.0	6.6 $\pm$ 3.7	7.1 $\pm$ 2.6	2.5 $\pm$ 3.2	4.8 $\pm$ 4.0	7.3 $\pm$ 3.5	8.4 $\pm$ 3.0	7.4 $\pm$ 2.7	8.7 $\pm$ 3.3	4.3 $\pm$ 3.8	6.5 $\pm$ 3.1	6.3 $\pm$ 3.9	6.9 $\pm$ 3.8
<i>Staphylococcus aureus</i> ATCC	9.1 $\pm$ 3.4	9.1 $\pm$ 2.4	6.8 $\pm$ 3.3	7.0 $\pm$ 3.3	4.9 $\pm$ 3.4	4.5 $\pm$ 4.2	8.1 $\pm$ 3.6	9.7 $\pm$ 5.5	8.3 $\pm$ 2.8	9.3 $\pm$ 2.4	5.6 $\pm$ 3.3	6.3 $\pm$ 3.4	6.2 $\pm$ 3.4	7.8 $\pm$ 3.1
<i>Yersinia enterocolitica</i>	9.6 $\pm$ 2.1	8.9 $\pm$ 1.9	6.3 $\pm$ 4.2	7.9 $\pm$ 3.4	9.9 $\pm$ 5.8	8.8 $\pm$ 3.3	9.5 $\pm$ 3.1	8.9 $\pm$ 2.7	10.4 $\pm$ 3.9	10.1 $\pm$ 3.5	7.1 $\pm$ 4.0	7.0 $\pm$ 3.3	7.7 $\pm$ 3.4	9.4 $\pm$ 2.8
Mean ZI	7.9 $\pm$ 4.0	8.3 $\pm$ 3.4	5.4 $\pm$ 3.6	5.5 $\pm$ 3.3	4.5 $\pm$ 3.9	4.8 $\pm$ 2.7	7.0 $\pm$ 3.8	7.8 $\pm$ 3.3	7.9 $\pm$ 3.7	8.1 $\pm$ 3.5	3.3 $\pm$ 3.5	4.5 $\pm$ 3.6	4.8 $\pm$ 3.5	6.2 $\pm$ 3.7

The values do not include the paper disc diameter. The coating thickness (low/high) is shown in parenthesis. MSE was from the 1st extraction with 80% acetone

combinations involving MSE were significantly less effective than the individual components ($p < 0.001$).

These results may reflect differences in the antimicrobial modes of action of the natural compounds. Some studies have shown that, in general, plant extracts are adsorbed to bacteria cell membranes, leading to disruption of the membrane and subsequent leakages of the cell contents (Zhu et al. 2004; Xu et al. 2008; Wang et al. 2010). However, in the present study, the modes of action of the natural compounds were not evaluated.

The average antibacterial effectiveness of the single-extract coatings directly reflected their TPC (TA > MSE > FSE) (Tables 1, 2). This order of results from extracts printed onto the film corresponds to the previously observed order of antibacterial effectiveness for these extracts dissolved in water (Widsten et al. 2014).

The relationship between antimicrobial activity and the type of bacteria was also evaluated. In general, Gram-negative bacteria were more resistant than Gram-positive bacteria (producing smaller ZIs on average) (Table 2), which is in agreement with a recent review (Faid 2011). TA-High was significantly more effective against Gram-positive than Gram-negative bacteria ($p < 0.0001$). It also proved effective against the Gram-negative bacteria *P. fluorescences* and *P. phosphoreum*, but the interaction was not significant ($p = 0.073$). The pattern was similar for TA-Low, but the effects were smaller and not significant ($p = 0.487$). Although the individual tested strains showed variable ZIs, the differences between them were not significant ($p = 0.968$).

Conclusion

It is concluded that the method of applying non-volatile phenolic-type antimicrobial compounds onto the surface of commercial films by flexographic printing has potential for the production of antimicrobial films. It was also confirmed that tannic acid presented a broad spectrum of antibacterial activity and was the most effective of the non-volatile antibacterial extracts investigated.

More studies need to be conducted to better understand the differences in the antimicrobial modes of action of the natural extracts.

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