EDIBLE COATINGS AND RETENTION OF POTASSIUM SORBATE ON APPLES, TOMATOES AND CUCUMBERS TO IMPROVE ANTIFUNGAL ACTIVITY DURING REFRIGERATED STORAGE

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ABSTRACT

Fresh apples, tomatoes and cucumbers were coated with guar gum (GG, 1.0% w/v), pea starch (PS, 4.0% w/v) or potato starch (PotS, 4.0% w/v) containing potassium sorbate (KS, 1.0% w/v) and stored at 4°C for 25 days. Coatings weight, thickness, KS surface concentration and yeast and mold counts were determined throughout the storage. GG coatings on apples and tomatoes produced the largest liquid film weight per unit area whereas after drying, PS coatings produced the largest coating weight and thickness. GG coatings deposited the largest initial KS surface concentration whereas PS coatings maintained KS at the surface and both coatings exhibited the greatest antifungal effectiveness. The relationships between KS surface concentration and the yeast and mold counts presented good correlations (R² between 0.75 and 0.90) and were linear for PS and PotS coatings and logarithmic for GG coatings indicating that multiple factors affecting depletion of KS from GG coatings.

PRACTICAL APPLICATIONS

Potassium sorbate (KS), in aqueous solution, has limited antifungal effectiveness when used for spraying and dipping of fresh fruits and vegetables. This limited effectiveness could result from quick depletion of KS from the surface of produce. Incorporating KS in edible coatings would reduce the quick depletion of KS, thus prolonging its antifungal activity. Determining the capability of different edible coatings to deposit and maintain KS on the surface of selected fruits and vegetables and correlating that to its antifungal activity could help in understanding the mechanism of inhibition of different coatings and selecting the suitable coating for each produce type.

INTRODUCTION

Contamination of fruits and vegetables with surface microorganisms is a major problem that leads to reduced shelf-lives of stored produce. Except for recommended washing, fresh fruits and vegetables are usually consumed without any treatment that reduces surface contamination (Moss 2008). There is a recent increase in consumer awareness for the safety of consuming fresh fruits and vegetables, as well as reducing environmental pollution that results from excessive waste generated from disposal of synthetic packaging materials (Ruban 2009; Janjarasskul and Krochta 2010).

Besides being able to carry a wide variety of functional ingredients such as antimicrobial compounds, antioxidants, flavoring and coloring compounds, edible coatings are considered environment friendly materials generated from natural resources (Daniel and Zhao 2007). Antimicrobials in packaging materials or edible coatings protect the functional antimicrobials from quick decomposition by limiting interactions with food and the atmosphere (Baldwin 1994).
Furthermore, packaging and edible coatings act as antimicrobial reservoirs, providing a continuous supply of the antimicrobial compounds within addressed time and maintain concentrations on the food surface greater than the minimum inhibitory concentrations (mic) for targeted microorganisms (Mehyar et al. 2007). Polysaccharide-edible coatings exhibit great potential for use in the food industry because they are inexpensive, compatible with a large range of functional compounds and present small allergy problems (Cha and Chinnan 2004; Daniel and Zhao 2007; Cho et al. 2009). Starch-based coatings containing selected organic oils are inhibitory to a large range of microorganisms (Ehivet et al. 2011; Kuorwel et al. 2011). Mehyar et al. (2011) reported that guar gum- (GG), pea starch- (PS) and potato starch- (PotS) edible coatings are suitable substrates to carry potassium sorbate (KS), improving the antifungal activity of KS against spoilage molds isolated from selected fruits and vegetables.

Effective antimicrobial coatings will wet and adhere to the surface of produce through low surface energy differences between the coatings and fruit surfaces (Hershko and Nussinovitch 1998; Choi et al. 2002). Antimicrobial coatings must also be thick enough to provide adequate concentration of the antimicrobial compound throughout the storage period (Ehivet et al. 2011). The adhering thickness of the coatings depend on viscosity, concentration and density of the biopolymer solution as well as draining time, chemical composition, surface properties of the coating solutions and the surfaces being coated (Cisneros-Zevallos and Krochta 2003). Furthermore, the release rate of KS from the coating depends on the type of chemical binding between KS and the biopolymer coating (Carlin et al. 2001; Kuorwel et al. 2011). Therefore, antifungal effectiveness of edible coatings is related to differences in chemical and physical characteristics (Arfa et al. 2007; Suppakul et al. 2011). Becerril et al. (2007) investigated several techniques that provide insight into the mechanisms of action of the active essential oils incorporated in active packaging and reported that direct contact assays in which the effect of antimicrobial compounds were determined by microbial growth was one of the most effective methods. Microbial growth was related to the amount of KS released from the coating surface in physical contact with the microorganisms. In another study, concentrations of antimicrobial were in physical contact with the target microorganisms at the surface of food in critical time determined the inhibitory effect of the antimicrobial (Gutiérrez et al. 2010). Therefore, the objective of the current research was to investigate the capabilities of GG, PS, and PotS coatings to deposit and maintain KS concentrations on the surface of apples, tomatoes and cucumbers. The second objective was to determine the protective extent of selected coatings to KS from depletion and maintain the antifungal activity by correlating KS surface concentration with antifungal effectiveness during refrigerated storage.

**MATERIALS AND METHODS**

**Preparation of the Antifungal Coating Solutions**

Aqueous suspensions of GG (1.0% w/v), PS (4.0% w/v) or PotS (4.0% w/v) were prepared in distilled water. Glycerol was added as plasticizer to the suspensions at concentration of 1:2 (glycerol : polymer; dry weight basis). The suspensions were heated on a hot plate with stirring to the boiling temperature (~105°C) and were held at the boiling temperature for 5 min to promote full gelation of the biopolymers. The solutions were cooled to room temperature and the pH of the polymer solutions adjusted to 4.5 with 1.0 N citric acid (Sigma-Aldrich, St. Louis, MO). KS (Sigma-Aldrich) was added to the prepared solutions at a concentration of 1.0% (w/v) and the coatings were homogenized with a high-speed homogenizer (CAT × 120, Ingenieurbüro CAT, Staufen, Germany) at 10,000 rpm for 1 min. Coating solutions were degassed in a vacuum (100 mbar) created by a vacuum pump (16,692, Sartorius, Goettingen, Germany) for 15 min. Self-standing films were prepared by casting 13 g of the coating solutions in high-density polyethylene plates and left overnight for drying at room temperature (25°C) and 40% relative humidity. Density of dried films were determined by measuring the weight and volume of the prepared films (Cisneros-Zevallos and Krochta 2003).

**Coating Application**

Intact apples, cucumbers and tomatoes were used in the experiments. Upon arrival at the laboratory, the fruit and vegetables were cleaned with sterilized cloth to remove physical dirt from the surface and used within 3 h of arrival time. Each produce’s population (150 produce) was split into five homogenous portions (30 produce). The first, second and third portions were used for dipping in one of the three coating solutions (GG, PS or PotS) containing KS for 15 s. The produce was drained in a stainless steel strainer and allowed to dry under aseptic conditions in a laminar flow cabinet for 0.6 h (for starch coatings) and for 1.3 h (for the GG coatings) at room temperature (25°C). The end of the drying period was determined when no change in the weight of produce was detected over the drying period. The fourth portion of produce was dipped for 15 s in KS aqueous solution (1.0%) prepared with sterilized water. The fifth portion was dipped for 15 s in sterilized distilled water as a control. The coated and control produce were placed in triplicates in sterile plastic bags and incubated at 4°C for 25
days and used to determine surface concentration of KS and yeast and mold counts.

**Determination of Coatings Weight and Thickness**

Intact apples, tomatoes and cucumber were weighed with a digital top-loading balance (PGL 20002, Adam Equipment, CT) with sensitivity of 0.01 g before and after dipping in the coating solutions and after the drying period. The weight (g) of the liquid film and dried coatings were determined by subtracting the weight of uncoated produce from the weight of the coated produce before and after drying, respectively. The percentage of the coating weight loss during drying was calculated as follows:

\[
\text{Percentage of the weight loss} = \left(1 - \frac{w_{\text{dried coating}}}{w_{\text{liquid film}}}ight) \times 100\%
\]

After the coating drying period, the produce was washed by tap water to remove the dried coatings from the surface then dried by placing in laminar flow for 30 min. The whole peel for each produce was removed with a sterile manual peeler that produced 1.5-mm thickness peels, which were collected and weighed. Pieces of peels with specific dimensions (10 × 3 cm) for each produce were weighed and used to calculate weight to surface area ratio that was calculated as 0.16, 0.20 and 0.21 g/cm² for apples, tomatoes and cucumbers, respectively. The surface area of the coated produce was calculated by using the weight of peels removed from the surface and the weight to surface area ratio. The weight of liquid films and dried coatings per unit area of produce was calculated by dividing the weight of liquid films and coatings on the calculated surface area.

Approximate thickness of the dried coatings was determined as follows:

\[
\text{Thickness (μm) of dried coating} = \frac{\text{weight of dried coating per unit area (g/cm²)}}{\text{density of the dried coating (g/cm³) × 10⁴}}
\]

The calculated amount of KS (μg/cm²) deposited on the surface of produce = weight of the liquid film per unit area (g/cm²)× concentration of KS in coating solution (1.0 g/100 g)×10⁶

\[
\text{(3)}
\]

**Assay of KS on Produce Surfaces**

The residual concentrations of KS on the produce surface were assayed by high-performance liquid chromatography (HPLC) according to Ferreira et al. (2000) with some modifications. Initially (within 1 h of drying) and throughout the incubation period, intact apples, tomatoes and cucumbers were peeled and 10 g of the peels were homogenized with 20 mL of the mobile phase (350 mL methanol and 650 mL acetate buffer [pH 4.52]) by using a high-speed homogenizer (CAT × 120 Ingenieurbüro). The mixture was filtered through Whatman No. 42 filter paper followed by membrane filtration (0.45 μm pore size) and degassed in an ultrasonic bath (UR1, Düsseldorf, Germany). An ODS C-18 column (150 × 4 mm) with a 5-μm particle diameter, which was used in the assay, which was conducted at room temperature. The HPLC was equipped with UV detector and set at 235 nm wave length and 0.7 mL/min flow rate. Standard curves of sorbic acid were created using external standards, and the smallest detection limit of the system was 10 μg/mL as the smallest KS concentration the system detected.

\[
\text{Percentage recovery of KS} = \frac{\text{assayed KS concentration (μg/cm²)}}{\text{calculated KS concentration (μg/cm²) × 10²}} \times 100\%
\]

\[
\text{(4)}
\]

**Yeast and Mold Counts during the Incubation**

Produce was withdrawn in triplicates from the incubator every 5 days and peels removed by a sterile manual peeler. The peels were homogenized with peptone water to prepare homogenate (10⁻¹). Aliquots from each serial dilution of the peel homogenate were spread on aerobic plate count agar (Oxoid Ltd., Cambridge, UK) containing chloramphenicol and chlorotetacycline HCL antibiotics (250 mg of each in 50 mL media) followed by incubation at 25C for 5 days (Tournas et al., 2001). The initial counts were determined by plating three peel homogenates directly after the coating procedure. The antifungal effectiveness of KS in edible coatings or in aqueous solutions was determined by comparing mold counts of treatments with the controls. Log₁₀ reductions were calculated by subtracting logarithms of yeast and mold counts of treated produce from logarithms of yeast and mold counts of the control at each storage time:

\[
\text{Log₁₀ reduction in yeast and mold counts} = \log₁₀ \left( \frac{\text{counts (log₁₀ cfu/g of treated produce)}}{\text{counts (log₁₀ cfu/g of control)}} \right)
\]

\[
\text{(5)}
\]

**STATISTICAL ANALYSIS**

The statistical analytical system version 8.2 (SAS institute Inc., Cary, NC) was used to compare treatment means and log reductions of microbial counts for each storage time. A significance level of \( P < 0.05% \) was selected for use. Means of the coating weight, KS residual concentration and yeast and mold counts were calculated from triplicate samples (\( n = 3 \)) and duplicate plating for the microbial counts (\( n = 6 \)) at each storage time.
RESULTS AND DISCUSSION

Table 1 presents densities of dried films, weight per unit area (g/cm²) of GG, PS and PotS liquid films and dried coatings. GG liquid films on apples and tomatoes exhibited significantly (P < 0.05) greater weights compared with the other films. PS coatings produced the second greatest liquid films per unit area, significantly (P < 0.05) greater than PotS liquid films on selected produce. The large liquid films weight may indicate desired adhesion and compatibility between the films and the produce (Casariego et al. 2008). Coating-solution properties such as viscosity may affect adhesion between the coatings and the surface of produce (Cisneros-Zevallos and Krochta 2003). The high viscosity of GG solution at a concentration of 1.0% (w/v) may be responsible for producing a thick liquid film on produce surfaces (Srichamroen 2007). PS contains large concentrations (35%) of amylose compared with PotS (Polesi et al. 2011). Amylose is compacted, binds water and swells during heating in presence of excess water, thus, responsible for increasing viscosity and formation of a thicker layer of coating film (Della Valle et al. 1996; Ratnayake et al. 2002). As presented in Table 1, GG coatings provided the smallest dried thickness and weight, but the largest density after drying. This is attributed to extensive water loss during the evaporation period related to small total solids content producing a thin but condensed coating. PS coatings produced the largest weight per unit area and the greatest thickness after drying, potentially a result of the combined effect of the large liquid film weight and lower percentage of weight loss (Table 1). Accordingly, during the drying process, the solids of PS liquid films are concentrated to produce a relatively thick layer (Table 1). PotS coatings exhibited the smallest liquid films weight before drying but comparable density with PS coatings and produced the intermediate dried coating weight and thickness (Table 1). The relatively low viscosity of PotS coatings that resulted from small amylose content resulted in the smallest liquid films weight. The small weight of PotS liquid films accompanied with great water evaporation rate produced dried coatings with weights smaller than PS coatings but larger than GG coatings. The larger percentage of weight loss during drying in GG coatings compared with PS and PotS coatings could be related to the lower polymer concentration in GG coatings (1.0%) compared with starch coatings (4.0%). Polymer may contribute in binding and retaining water during drying of polymer coatings producing lower percentage of loss (Table 1).

Table 2 presents initial surface concentrations of KS (assayed and calculated) as well as the percentage recoveries. GG coatings produced the largest (P < 0.05) initial KS surface concentrations on apples and tomatoes, followed by PS and PotS coatings. The large dried coating density of the GG coatings may indicate the GG coatings are condensed, and the largest KS concentration is expected in the GG coatings (Table 1). The other coatings, in particular the PS coatings followed by PotS coatings, were less effective in depositing KS on the produce surfaces (Table 2). Both starch coatings exhibited similar dried densities but different in dried coating thickness (Table 1). PS coatings deposited larger concentration of KS, which could be attributed to the larger coating thickness after drying (Table 1). KS concentrations were smallest with the smallest percentage recovery on cucumbers than on apples and tomatoes for all coating types. This could be related to the greater surface roughness of cucumber. The greater roughness of cucumbers could indicate presence of large pores on the surface that may help in quicker absorption and interaction of the

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Density of dried film (g/cm³)</th>
<th>Weight of liquid film/area (g/cm²)</th>
<th>Weight of dried coating/area (g/cm²)</th>
<th>Percentage of weight loss (%)</th>
<th>Thickness of dried coating (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG coating</td>
<td>0.313 ± 0.032</td>
<td>0.0508 ± 0.0083</td>
<td>0.0011 ± 0.0009</td>
<td>97.9</td>
<td>35 ± 5</td>
</tr>
<tr>
<td>Apples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomatoes</td>
<td></td>
<td>0.0519 ± 0.0021</td>
<td>0.0012 ± 0.0005</td>
<td>97.7</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>Cucumbers</td>
<td></td>
<td>0.0464 ± 0.0056</td>
<td>0.0010 ± 0.0002</td>
<td>97.8</td>
<td>32 ± 4</td>
</tr>
<tr>
<td>PS coating</td>
<td>0.115 ± 0.007</td>
<td>0.0423 ± 0.0042</td>
<td>0.0039 ± 0.0005</td>
<td>90.9</td>
<td>339 ± 21</td>
</tr>
<tr>
<td>Apples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomatoes</td>
<td></td>
<td>0.0403 ± 0.0091</td>
<td>0.0041 ± 0.0006</td>
<td>89.9</td>
<td>356 ± 35</td>
</tr>
<tr>
<td>Cucumbers</td>
<td></td>
<td>0.0416 ± 0.0074</td>
<td>0.0033 ± 0.0004</td>
<td>92.0</td>
<td>287 ± 18</td>
</tr>
<tr>
<td>PotS coating</td>
<td>0.157 ± 0.009</td>
<td>0.0204 ± 0.0015</td>
<td>0.0021 ± 0.0002</td>
<td>89.8</td>
<td>134 ± 11</td>
</tr>
<tr>
<td>Apples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomatoes</td>
<td></td>
<td>0.0252 ± 0.0008</td>
<td>0.0022 ± 0.0006</td>
<td>91.1</td>
<td>140 ± 18</td>
</tr>
<tr>
<td>Cucumbers</td>
<td></td>
<td>0.0206 ± 0.0032</td>
<td>0.0019 ± 0.0004</td>
<td>90.7</td>
<td>121 ± 8</td>
</tr>
</tbody>
</table>

Values within the same column with different superscripts are significantly different (P < 0.05).

* Means ± standard deviation (n = 3).

GG, guar gum; PS, pea starch; PotS, potato starch.
TABLE 2. INITIAL SURFACE CONCENTRATIONS (ASSAYED AND
CALCULATED) AND PERCENTAGE RECOVERIES OF KS ON APPLES,
 TOMATOES AND CUCUMBERS COATED WITH EDIBLE COATINGS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>KS concentration (µg/cm²)</th>
<th>Assayed</th>
<th>Calculated</th>
<th>Percentage recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG coating</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apples</td>
<td>502.69ab</td>
<td>507.95a</td>
<td>98.96</td>
<td></td>
</tr>
<tr>
<td>Tomatoes</td>
<td>489.66bc</td>
<td>519.13a</td>
<td>94.32</td>
<td></td>
</tr>
<tr>
<td>Cucumbers</td>
<td>398.67cd</td>
<td>464.11b</td>
<td>85.90</td>
<td></td>
</tr>
<tr>
<td>PS coating</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apples</td>
<td>409.46c</td>
<td>422.89a</td>
<td>96.83</td>
<td></td>
</tr>
<tr>
<td>Tomatoes</td>
<td>393.59cd</td>
<td>402.78b</td>
<td>97.72</td>
<td></td>
</tr>
<tr>
<td>Cucumbers</td>
<td>352.66de</td>
<td>415.73c</td>
<td>84.83</td>
<td></td>
</tr>
<tr>
<td>PotS coating</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apples</td>
<td>221.62d</td>
<td>232.59f</td>
<td>95.28</td>
<td></td>
</tr>
<tr>
<td>Tomatoes</td>
<td>239.64e</td>
<td>252.15e</td>
<td>95.04</td>
<td></td>
</tr>
<tr>
<td>Cucumbers</td>
<td>172.59f</td>
<td>206.43d</td>
<td>83.61</td>
<td></td>
</tr>
</tbody>
</table>

Values within the same column with different superscripts are significantly different (P < 0.05).

KS, potassium sorbate; GG, guar gum; PS, pea starch; PotS, potato starch.

KS contained in the coatings with the cucumber surfaces (Quevedo and Aguilera 2004).

Table 3 presents residual concentrations (µg/cm²) of KS on apples, tomatoes and cucumbers coated with GG, PS and PotS or treated with KS in aqueous solution throughout the studied incubation period. The selected edible coatings enhanced retention of KS compared with KS without a coating treatment throughout the testing period. When used without coating, the KS concentration was reduced to a concentration less than the detection limit at the tenth day of incubation, whereas PS coatings retained KS surface concentrations greater than the detection limit for 25 days on apples and 20 days on cucumbers and tomatoes. GG coatings exhibited relatively less effectiveness than PS coatings in retaining KS surface concentrations (Table 3). GG coatings may provide less protection resulting in quick depletion of the large initial KS surface concentrations explained previously (Table 2). The small thickness of GG coatings may result in large quantities of KS are exposed to the environment and produce’s surface and inactivated by interactions with their components (Arfa et al. 2007). PotS coatings were the least effective in retaining KS surface concentrations. PotS coatings retained KS at smaller concentrations and for shorter times compared with the GG and PS coatings (Table 3). This could be resulted from incapability of these coatings to deposit larger initial concentration of KS (Table 2). The ineffectiveness of PotS coatings confirms previous findings that antimicrobial films lose activity through depletion of antimicrobial compounds (Suppakul et al. 2011). The use of lower storage temperatures than used in the current study (4C) may contribute to slower diffusion and interaction of KS in GG and PotS coatings, thus longer retention of surface KS and better fungal inhibition (Arfa et al. 2007; Suppakul et al. 2011).

Figure 1 presents reductions in the counts of yeast and mold on the surface of the coated and uncoated produce during the incubation period. The average level of natural yeasts and molds on the test product was 3.7 × 10³ cfu/g. Incorporation of KS with the selected coatings significantly (P < 0.05) improved antifungal effectiveness on apples, tomatoes, and cucumbers when compared with KS in aqueous solutions (Fig. 1). The inhibition of yeast and mold by KS in GG and PS coatings were comparable during the first 15 days of storing apples and tomatoes. However, the PS coatings resulted in longer inhibition (Fig. 1). KS in the PotS coatings applied on cucumbers was the least effective inhibitor of yeast and mold. Differences in inhibition of yeast and mold by KS in edible coatings may be attributed to different retention of intact KS concentration by different coatings greater than the mic of the targeted fungi (Daniel and Zhao 2007). For example, the greatest yeast and mold growth inhibition was obtained by KS in the PS coatings (2.2 log10 cfu/g) on day 20 of incubation on apples, and the KS surface concentration was also the largest (271.1 µg/g) on day 20 of incubation. The selected coatings were more inhibitory on apples and tomatoes compared with cucumbers. The greatest inhibition of yeast and mold were 2.2 log10 cfu/g and 1.7 log10 cfu/g obtained on apples and tomatoes, respectively, whereas the greatest reduction on cucumbers was 1 log10 cfu/g after 10 days. The surface roughness of cucumbers may reduce effectiveness of KS in starch coatings through enhancing microbial attachment (Mehyar et al. 2011).

TABLE 3. RESIDUAL CONCENTRATIONS (µg/cm²) OF KS ON APPLES,
 TOMATOES AND CUCUMBERS COATED WITH SELECTED EDIBLE
 COATINGS OR TREATED WITH KS IN AQUEOUS SOLUTION DURING
 REFRIGERATED STORAGE

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>GG coating</td>
<td></td>
</tr>
<tr>
<td>Apples</td>
<td>452.1</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>431.4</td>
</tr>
<tr>
<td>Cucumber</td>
<td>324.5</td>
</tr>
<tr>
<td>PS coating</td>
<td></td>
</tr>
<tr>
<td>Apples</td>
<td>403.8</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>363.6</td>
</tr>
<tr>
<td>Cucumber</td>
<td>311.5</td>
</tr>
<tr>
<td>PotS coating</td>
<td></td>
</tr>
<tr>
<td>Apples</td>
<td>198.3</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>206.8</td>
</tr>
<tr>
<td>Cucumber</td>
<td>142.8</td>
</tr>
<tr>
<td>KS in aqueous solution</td>
<td>109.5</td>
</tr>
</tbody>
</table>

KS, potassium sorbate; GG, guar gum; PS, pea starch; PotS, potato starch; UD, undetected.
Coating thickness, adhesion, continuity and the KS release rate are important factors in determining fungi inhibition of the antimicrobial edible coatings (Carlin et al. 2001). The degree of biopolymer matrix cross-linking, swell-ability, the surrounding relative humidity, and temperature will affect the release kinetics of antimicrobial compounds (Papadokostaki et al. 1997; Buonocore et al. 2004). The initial antifungal effectiveness of KS in GG coatings may be related to the large initial KS surface concentration released by GG coatings. Whereas, PS coatings retain the larger KS surface concentrations and antifungal activity for longer times (20 days). Retention of KS surface concentration by PS coatings probably occurs through controlled release of KS and protection from decomposition that could be related to larger coating thickness (Tables 1 and 3). The prolonged antifungal effectiveness of PS coatings compared with GG and PotS coatings against spoilage molds inoculated onto apples, tomatoes and cucumbers was previously demonstrated under similar experimental conditions (Mehyar et al. 2011).

The relationship between the residual concentration of KS and the reduction of yeast and mold counts was increasing and is logarithmic for GG coatings and linear for PS and PotS coatings with correlation ($R^2$) between 0.75 and 0.90 (Fig. 2). KS surface concentrations are directly proportional to yeast and mold counts reduction when present in PS and PotS coatings. Growth inhibition indicates KS is released on the produce surface, and suggests KS present in PS and PotS coatings is accessible for yeast and mold inhibition. The steeper slope of PotS curve with concentrations of KS retained at less than 200 mg/cm² on apples and tomatoes indicates that PotS coatings quickly release KS to the surface and produce relatively better initial fungal inhibition than the PS coatings (Fig. 2). The relationship between KS concentration and inhibition with the GG coatings was logarithmic with the KS concentrations on the produce surface comparable to KS concentrations retained by PS coatings (Fig. 2). The GG coatings produced the largest initial KS surface concentrations (Table 2), however, the logarithmic relationship indicates that other factors affected the depletion of KS since the depletion rate was not concurrent with reduction in its antimicrobial activity. KS may interact quickly with the surrounding components of the environment and result in poor retention of KS by GG coatings.

**FIG. 1. REDUCTIONS IN YEAST AND MOLD ON APPLES (A), TOMATOES (B) AND CUCUMBERS (C) COATED WITH SELECTED EDIBLE COATINGS DURING REFRIGERATED STORAGE.** Different letters within the same sampling time indicate significant differences ($P < 0.05$). GG, guar gum; PS, pea starch; PotS, potato starch.
It is expected that KS exhibits limited migration through the GG coatings because of the large density of the GG coatings after drying (Table 1). Accordingly, the GG coatings may possess antifungal activity from the KS presented initially on the surface, which may explain previous findings of the capability of the GG coatings to reduce yeast and mold counts initially with little prolonged antifungal activity (Fig. 1). Selection of experimental conditions such as storage temperature, relative humidity, time, produce and fungi strains may affect antifungal effectiveness of KS. The high relative humidity conditions, larger water content of produce, and high temperature may improve flexibility and mobility of the long hydrophilic polymer strands and diffusivity of hydrophilic solutes such as KS (Arfa et al. 2007).

CONCLUSION

The differences in the antifungal activity of selected coatings containing KS were assessed by determining coating adherence and thickness on produce surfaces. GG, PS and PotS coatings retained KS concentrations on the surface of produce to effectively provide antifungal activity and protect KS from disappearance during refrigerated storage.

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