



Research article

Development of antimicrobial coating from tapioca starch incorporated with organic salt and acetic acid and its effect on cucumber quality

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Abstract

The application of an antimicrobial coating is one of the alternative approaches for post-harvest treatment to extend the shelf-life of perishable vegetables and fruits. A coating was investigated developed from tapioca starch incorporated with organic salts (sodium lactate and potassium sorbate) and acetic acid for its effect on microbial inhibition on cucumbers. The results showed that the low pH of the developed coating containing acetic acid could increase the efficiency of microbial inhibition activity. The inhibition zones of each studied microorganism (*Escherichia coli*, *Staphylococcus aureus* and *Aspergillus* sp.) were significantly ($p < 0.05$) different. The antimicrobial coating of tapioca starch containing the mixture of sodium lactate potassium sorbate and acetic acid had the best antimicrobial activity as a result of synergy in the preservative combination. The surface application of the developed antimicrobial coating improved the quality of cucumber with little effect on color acceptance after storage for 3 d. Generally, the coated cucumbers received better sensory scores than control uncoated cucumbers. The coating of tapioca starch resulted in a greater reduction in *E. coli* inoculation on cucumbers than for the other pathogens, indicating that this antimicrobial coating can be used for pathogen inhibition, specifically *E. coli*.

Introduction

Cucumber has caused disease worldwide as it is preferably eaten fresh without heating. Its shelf-life is short, which results in economic losses because of chemical and thermal limitations (Raghav and Saini, 2018). Several foodborne pathogens—*Salmonella* Typhimurium, *Staphylococcus aureus*, *Lactobacillus plantarum*, and *Listeria monocytogenes*—are commonly found on the skin of cucumbers (Reina et al., 2002) and reducing pathogenic contamination in cucumbers is challenging. The current trend by consumers demanding

fresh, clean and safe vegetables means that the food industry must find solutions to meet this demand, by producing products having microbial safety and minimal processing (Patel and Panigrahi, 2019). The concepts of minimal processing and hurdle technology are of interest to minimize the growth of pathogens in perishable foods for post-harvest strategies.

An antimicrobial coating, known as a hurdle-base method, is being increasingly developed to improve the quality and shelf-life of perishable vegetables and fruits; its base component can be produced from protein, carbohydrate and fats, which are mostly mixed with glycerol as a plasticizer. As most ingredients used in coating solutions are categorized as Generally Recognized as Safe (GRAS) materials, the coated food product is safe for human consumption (Sonti, 2003; Kramer, 2009). However, the means of protection can have

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different effects on food products (Dangaran et al., 2009), including fat retarding water migration of products, polysaccharide preventing gas exchange on the skins of products and protein improving the appearance and texture of products (Mehyar et al., 2012).

The coating solution can have several functions by the addition of food additives, antioxidants and antimicrobials, which depends on the purpose of the coating application (Raghav and Saini, 2018). The selection of ingredients for inhibiting pathogens depends on the targeted species of pathogen as well as the type of applied products (Patel and Panigrahi, 2019). Ingredients used include essential oils, chitosan, organic acids and organic salts, which work effectively at different temperatures and pH levels. Valencia-Chamorro et al. (2011) reported that lactic acid, sodium lactate and potassium sorbate had antimicrobial activity when used in coating production. Shen et al. (2010) also showed that adding potassium sorbate in sweet potato starch film could inhibit the growth of *E. coli* and *S. aureus*. Study of the specific effects of each coating type using mixtures of organic acids and organic salts in the coating formulation is still limited; therefore, it is important that the effect is also studied of the ingredients incorporated, in order to extend the coating application to other vegetables and foods for protection against various microorganisms.

The present study aimed to develop an antimicrobial coating by the addition of sodium lactate, potassium sorbate and acetic acid in tapioca starch solution and to investigate the effect of the developed antimicrobial coating on pathogen inhibition and the shelf-life of cucumbers. The results of the study could provide an alternative for post-harvest treatment using the antimicrobial coating to improve the quality of cucumbers and sensory evaluation and to also instantly kill bacteria prior to consumption.

Materials and Methods

Coating preparation

Tapioca starch from SCG Trading (Bangkok, Thailand) was dispersed in a 2.5% (weight per weight; w/w) glycerol water solution to obtain 0.5% (w/w) suspensions, which were heated on a hotplate set at 70°C for 30 min under stirring to accomplish complete starch gelatinization (Flores et al., 2007). The solutions were called tapioca starch solution (TSS) without adding organic acid and acetic acid. Other suspensions were prepared with a similar composition but by adding 1.34% sodium lactate (Bingol and Boston, 2007), 0.33% potassium sorbate (Flores et al., 2007), or 5% acetic acid (Campos et al., 2011) or combinations into tapioca starch solution. The seven obtained coating formulations were: sodium lactate (S), acetic acid (A), potassium sorbate (P), sodium lactate + acetic acid (SA), sodium lactate + potassium sorbate (SP), potassium sorbate + acetic acid (PA), sodium lactate + potassium sorbate + acetic acid (SPA). Thereafter, the pH and water solubility were measured.

Bacterial strain and preparation

E. coli DMST 4212, *S. aureus* DMST8840 and *Aspergillus* sp. IFRPD 4041 were obtained for use from the Food Quality Assurance Center, Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand. All strains were stored at -80°C in glycerol 40% (w/w). Before use, they were grown twice in tryptic soy broth (TSB; Merck; Germany) at 37°C for 24 hr.

Film disk agar diffusion assay

Film disk agar diffusion assay on solid agar was performed using *E. coli*, *S. aureus* and *Aspergillus* sp., according to the method in Vasconez et al. (2009). The *E. coli* and *S. aureus* strains were grown on TSB at 37°C for 6 hr. The *Aspergillus* sp. strain was grown on nutrient broth (NB; Merck; Germany) at 30°C for 72 hr. Then, 10 µL of inoculum were pipetted in 100 mL of nutrient agar (NA; Merck; Germany) and 10 mL of agar was poured on each plate. Afterward, the solid agar was punched with 6 mm diameter discs using a sterile cork borer. Then, 50 µL of coating solution was pipetted into each hole in the NA. *E. coli* and *S. aureus* were incubated at 37°C for 24 hr and the *Aspergillus* sp. strain was incubated at 30°C for 72 hr. After incubation, each diameter of the inhibition zone (clear area of no growth) was measured. A control coating without antimicrobial ingredients was also evaluated. The best results of the inhibition test using the three formulas were selected for coating cucumbers in the next steps.

Coating application on cucumbers

Cucumbers (*Cucumis sativus* L.) were obtained from a local market and depurated with water once. Cucumbers of the same size and with unblemished skin were manually selected by hand and dried at room temperature and the volume of each cucumber was determined using water displacement. For each storage temperature (25°C and 4°C), the good quality of cucumbers were divided into 16 treatments regarding the type of microorganisms (total bacteria, *E. coli*, *S. aureus* and *Aspergillus* sp.) for the control treatment (uncoated cucumber; CON) and for coating formulas (coated cucumber; SA, SPA and PA).

E. coli and *S. aureus* cultures in TSB and *Aspergillus* sp. cultures in NB were prepared at 37°C for 6 hr and 30°C for 72 hr, respectively, prior to use in the experiment, reaching a 1×10^6 colony forming units (cfu)/mL concentration. The cucumbers in each treatment were inoculated with each culture by dipping and were then left to dry for 10 min. This method was modified from Durango et al. (2006).

For each treatment, 10 mL of the coating solutions were sprayed and brushed on the surfaces of eight cucumbers (400 mL), which had been inoculated with suspension and then were dried for 15 min. The cucumbers were checked for the initial number of bacteria and then placed in a polyester plastic bag and stored at either 25°C or 4°C for 12 d. Cucumbers in plastic bags were randomly sampled every 3 d. The results of storage were compared between the coated

cucumbers and uncoated cucumbers. The same procedures were performed on all three formulas.

Microbiological analysis

For microbial enumeration, 10 g of each sample were aseptically transferred into a sterile stomacher bag containing sterile 0.85% NaCl solution (90 mL) to create a 1:10 dilution and after mixing, serial dilutions were prepared in 9 mL of sterile 0.85% NaCl solution in test tubes. A sample with 0.1 mL of each dilution was spread plated on the appropriate medium: plate count agar (Merck; Germany) for total bacteria, Baird-Parker medium containing egg yolk tellurite (Merck; Germany) for *S. aureus* (Bennet and Lancette, 1998), eosin methylene blue (Merck; Germany), after lauryl tryptose broth (Merck; Germany) and *Escherichia coli* (Merck; Germany) broth growth, respectively, for *E. coli* (Feng et al., 2002) and potato dextrose agar + tartaric acid 10% (Merck; Germany) for *Aspergillus* sp. (ISO 7954, 1987). Bacteria plates were incubated at 37°C for 24 hr, whereas mold plates were incubated at 30°C for 72 hr. Total counts of each pathogen were determined and converted to log cfu/g.

Sensory evaluation

The best results of coating solution were selected for sensory analysis using a 9-point hedonic scale to evaluate overall liking, flavor, aroma, texture and appearance using 30 untrained panelists. The results were compared between coated cucumbers and uncoated cucumbers during storage for 3 d.

Statistical Analysis

Data were subjected to analysis of variance facilitated by SPSS for Windows version 12 (SPSS Thailand Co., Ltd; Bangkok, Thailand) and comparison of means was carried out using Duncan's multiple range test. Differences among means in the numbers of microorganisms for each treatment were considered significant at $p \leq 0.05$.

Results

Effect of tapioca starch-organic salts-acetic acid on film disk agar diffusion assay

The developed coating from tapioca starch incorporated with organic salt (sodium lactate and potassium sorbate) and acetic acid was investigated for its microbial inhibition on *E. coli*, *S. aureus* and *Aspergillus* sp. Generally, all coating formulas had similar chemical and physical characteristics for pH values (1.72–5.25) and water solubility (0.11 and 0.18%). Low pH levels were recorded for the developed coatings containing acetic acid such as coating A (pH 1.72), coating SA (pH 2.94), coating PA (pH 2.61) and coating SPA (pH 3.01). The solubility of coating SPA was the lowest compared to the other tested formulas. The results of screening antimicrobial activities of the developed coating formulas showed that the inhibition zones of *E. coli*, *S. aureus* and *Aspergillus* sp., were significantly different among the developed coating formulas as shown in Table 1. The tapioca starch solution (TSS) did not show any inhibitory effect on any of the tested pathogens. Coating SA, coating SPA and coating PA had wide inhibition zones and so the antimicrobial effect was further investigated on cucumbers stored at 4°C and ambient temperature of 25°C for 12 d.

Decontamination of cucumbers coated with tapioca starch-organic salts-acetic acid

The antimicrobial activity of the developed coating from tapioca starch incorporated with organic salt and acetic acid was studied on total bacteria (total plate count) and three pathogenic species (*E. coli*, *S. aureus* and *Aspergillus* sp.) *in vivo*. The surfaces of cucumbers were inoculated with populations (1×10^6 cfu/mL) of the three pathogens and subsequently the microbial reductions between coated (SA, SPA, and PA) and uncoated (CON) cucumbers were compared during storage at the two different temperatures (4°C and 25°C).

Table 1 Inhibition zone of different coating on *E. coli*, *S. aureus* and *Aspergillus* sp.

Coating	Inhibition zone diameter (cm)		
	<i>E. coli</i>	<i>S. aureus</i>	<i>Aspergillus</i> sp.
Tapioca starch solution (TSS)	0.0±0.0 ^c	0.0±0.0 ^d	0.0±0.0 ^d
Sodium lactate (S)	0.0±0.0 ^c	2.0±0.0 ^b	1.5±0.1 ^{ab}
Acetic acid (A)	0.2±0.0 ^{bc}	0.0±0.0 ^d	0.0±0.0 ^d
Potassium sorbate (P)	0.1±0.1 ^{bc}	2.1±0.1 ^{ab}	1.2±0.1 ^c
Sodium lactate + Acetic acid (SA)	0.3±0.0 ^{abc}	2.4±0.1 ^a	1.6±0.1 ^a
Sodium lactate + Potassium sorbate (SP)	0.2±0.3 ^{bc}	1.5±0.1 ^c	1.3±0.1 ^{bc}
Potassium sorbate + Acetic acid (PA)	0.3±0.0 ^{ab}	1.7±0.1 ^c	1.4±0.1 ^{bc}
Sodium lactate + Potassium sorbate + Acetic acid (SPA)	0.5±0.1 ^a	2.1±0.2 ^{ab}	1.6±0.1 ^a

Values are mean ± SD of two replicates

Values with different lowercase superscripts in a column are significantly different ($p < 0.05$)

At 4°C, the microbial profiles of the inoculated cucumbers were significantly different between before and after the coating application and storage for 1 d. During 12 d of storage, the decontamination of bacteria cucumbers did not differ among all coating formulas stored at 4°C. For total bacteria, coating PA on cucumbers caused a 1.5 log cfu/g reduction on total plate counts, while coating SPA and coating SA reduced contaminated microorganisms by 0.5 log cfu/g and 0.8 log cfu/g, respectively, during the 12 d of storage (Fig. 1).

All coating formulas reduced *E. coli* numbers by 3.5 log cfu/g after coating and during the 12 d of storage (Fig. 2). Similarly, coating SA reduced *S. aureus* numbers by 1 log cfu/g (Fig. 3). The lowest *S. aureus* count was for coating SPA. For *Aspergillus* sp. (Fig. 4), the numbers on coated cucumbers reduced by 2 log cfu/g, which was lower than for uncoated cucumbers. Interestingly, *Aspergillus* sp. numbers remained stable for the rest of the storage period.

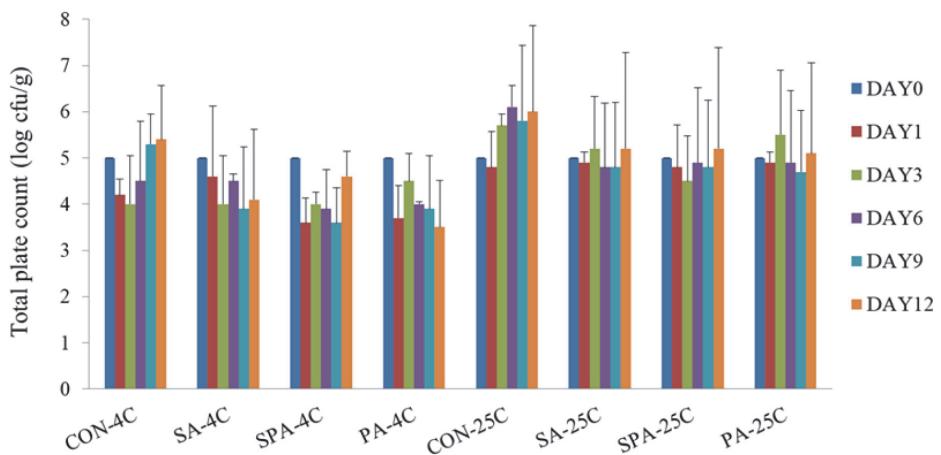


Fig. 1 Survival of total bacteria inoculated on coated and uncoated cucumbers ($n = 4$) stored at 4°C (4C) and 25°C (25C) during 12 d storage (6 sampling days), where values are mean \pm SD of three replicates, there were no significant differences ($p > 0.05$) in survival numbers during storage; CON = uncoated cucumber; SA = cucumbers with coating containing sodium lactate-acetic acid; SPA = cucumbers with coating containing sodium lactate-potassium sorbate-acetic acid; PA = cucumbers with coating containing potassium sorbate-acetic acid; cfu = colony forming units

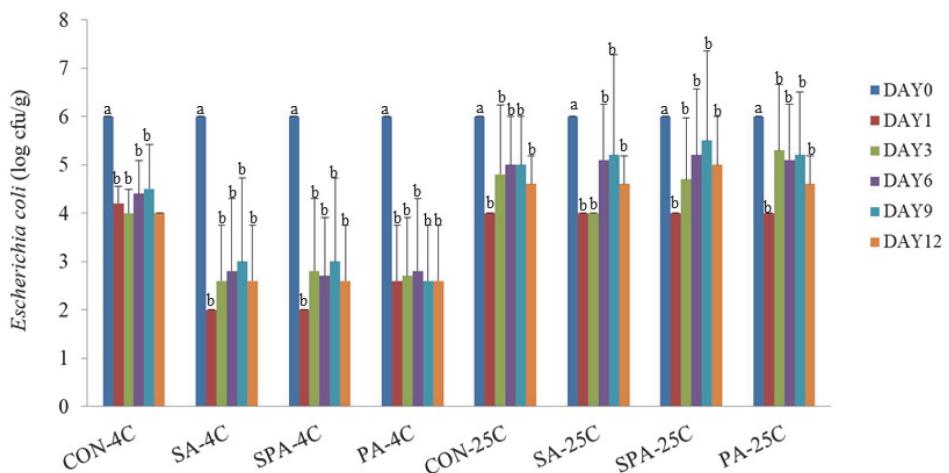


Fig. 2 Survival of *Escherichia coli* inoculated on coated and uncoated cucumbers ($n = 4$) stored at 4°C (4C) and 25°C (25C) during 12 d storage (6 sampling days) where values are mean \pm SD of three replicates, different lowercase letters above columns indicate significant differences ($p < 0.05$) in survival numbers during storage; CON = uncoated cucumber; SA = cucumbers with coating containing sodium lactate-acetic acid; SPA = cucumbers with coating containing sodium lactate-potassium sorbate-acetic acid; PA = cucumbers with coating containing potassium sorbate-acetic acid; cfu = colony forming units

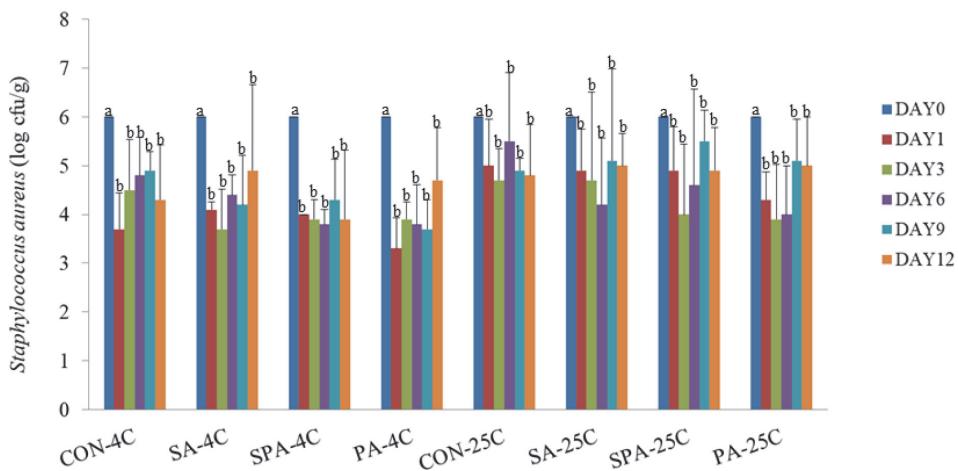


Fig. 3 Survival of *Staphylococcus aureus* inoculated on coated and uncoated cucumbers ($n = 4$) stored at 4°C (4C) and 25°C (25C) during 12 d storage (6 sampling days), where values are mean \pm SD of three replicates; different lowercase letters above columns indicate significant differences ($p < 0.05$) in survival numbers during storage; CON = uncoated cucumber; SA = cucumbers with coating containing sodium lactate-acetic acid; SPA = cucumbers with coating containing sodium lactate-potassium sorbate-acetic acid; PA = cucumbers with coating containing potassium sorbate-acetic acid; cfu = colony forming units

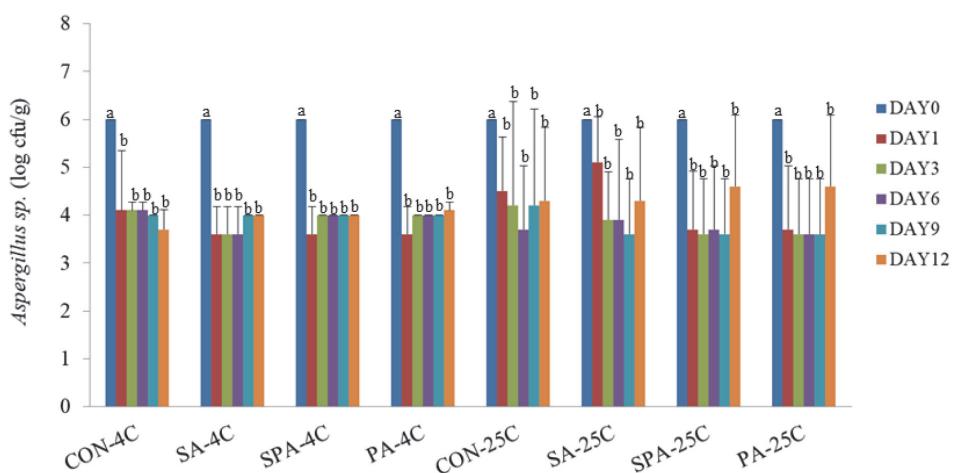


Fig. 4 Survival of *Aspergillus* sp. inoculated on coated and uncoated cucumbers ($n = 4$) stored at 4°C (4C) and 25°C (25C) during 12 d storage (6 sampling days), where values are mean \pm SD of three replicates, different lowercase letters above columns indicate significant differences ($p < 0.05$) in survival numbers during storage; CON = uncoated cucumber; SA = cucumbers with coating containing sodium lactate-acetic acid; SPA = cucumbers with coating containing sodium lactate-potassium sorbate-acetic acid; PA = cucumbers with coating containing potassium sorbate-acetic acid; cfu = colony forming units

At ambient temperature (25°C), all coating formulas resulted in significantly different microbial profiles of the inoculated cucumbers before and after application. Cucumbers with and without coating showed no significant differences on inhibition effect of all tested microorganisms during 12 days of storage. The microbial profiles of all treatments decreased immediately from the first day of storage with minor fluctuations. The numbers of total bacteria on coated cucumbers slightly increased but was lower than the uncoated cucumbers where values increased by 1 log cfu/g (Fig. 1). Similarly, the inoculated *E. coli* on cucumbers differed significantly between before and after coating (Fig. 2). The number of *E. coli* fell substantially after the

application of the developed antimicrobial coating before growing 0.7–1.3 log cfu/g again after 1 d of storage. The coated and uncoated cucumbers had slightly lower numbers of *S. aureus* during 3 d of storage (Fig. 3). After day 9, the bacterial levels in all treatments slightly declined to about the same numbers. The numbers of *Aspergillus* sp. were not significantly different between coated and uncoated cucumbers stored at 25°C (Fig. 4). After a coating application, the number of *Aspergillus* sp. inoculated on cucumbers reduced by 2.3 log cfu/g and then slightly increased at the end of storage. Generally, all coatings had similar effects on *Aspergillus* sp. inhibition.

Sensory evaluation on cucumbers coated with tapioca starch-organic salts-acetic acid

Thirty panelists evaluated five sensory attributes (appearance, color, flavor, texture and overall liking) for the four samples including the control using a 9-point hedonic scale (Table 2). The scores were significantly different for the appearance and color attributes of cucumbers with and without coating. The coating SPA received the highest mean score for cucumber color (6.2 ± 0.1) while the CON cucumbers had the lowest mean score (5.7 ± 0.0). The appearance and texture of cucumbers coated with the coating PA had the highest scores compared to the CON cucumbers. Generally, all coated cucumbers received higher scores compared with uncoated cucumbers and the least liking from the sensory evaluation was for uncoated cucumbers which were rated at 5.9 as “like slightly”. After 3 d of storage, the scores for cucumber color were different, with the SA cucumbers having the highest score (6.8), compared to SPA cucumbers with the lowest (6.6) as shown in Table 3. All coated cucumbers received similar scores to uncoated cucumbers. The average of each attribute was approximately 6.5, “like slightly”. The least liking from the sensory evaluation was for coating SPA (6.6), whereas uncoated cucumbers received the highest score for overall liking (7.1) though there was significant difference.

Discussion

The developed coatings from tapioca starch incorporated with organic salt (sodium lactate and potassium sorbate) and acetic acid were investigated for microbial inhibition on cucumbers.

The antimicrobial coating of tapioca starch containing sodium lactate, potassium sorbate and acetic acid (SPA) contributed the best inhibitory effect on the three pathogens (*E. coli*, *S. aureus* and *Aspergillus* sp.) using film disk agar diffusion, thereby being the best formula for decontamination of cucumbers. After a coating application, the developed coating was effective at reducing the numbers of *E. coli*, *S. aureus* and *Aspergillus* sp. on cucumbers, although there were no significant differences with storage time. However, the numbers of total bacteria and of the three pathogens (*E. coli*, *S. aureus* and *Aspergillus* sp.) inoculated on coated cucumbers were considerably lower than on uncoated cucumbers during storage. Although application of the developed coating affected the scores of color attributes among cucumbers after surface application and 3 d of storage, almost all acceptance scores of the coated cucumbers were higher than for the uncoated cucumbers.

Polysaccharide-edible coating is a promising method for surface decontamination of cucumbers since it is inexpensive and applicable over a large range of edible commodities (Mehyar et al., 2012). The incorporation of several substances such as chitosan and oil (Taştan et al., 2017), herbal extract and organic acid (Valencia-Chamorro et al., 2011) have been used to improve the efficiency of edible coatings. The degree of biopolymer matrix cross-linking, coating thickness, swell-ability, pH and adhesion of the polysaccharide are important factors regarding the release kinetics of antimicrobial compounds in determining pathogen inhibition (Mehyar et al., 2012). The current study found that a low pH level in the developed coating containing acetic acid increased the efficiency of microbial inhibition activity on film disk agar diffusion assay, as bacterial cells had greater sensitivity to the acidic coating solution than to the more

Table 2 Sensory evaluation of uncoated and coated cucumbers

Attributes	Coating formula			
	CON	SA	SPA	PA
Appearance	5.3 ± 0.0^b	5.9 ± 0.1^a	6.0 ± 0.1^a	5.8 ± 0.1^a
Color	5.7 ± 0.0^c	5.9 ± 0.1^b	6.2 ± 0.1^a	5.9 ± 0.1^b
Flavor	5.7 ± 0.3^a	5.8 ± 0.3^a	6.5 ± 1.9^a	5.7 ± 0.3^a
Texture	5.4 ± 0.4^b	5.7 ± 0.2^{ab}	5.9 ± 0.7^{ab}	6.2 ± 0.2^a
Overall liking	5.9 ± 0.6^a	6.0 ± 0.2^a	7.2 ± 1.7^a	6.3 ± 0.2^a

CON = uncoated cucumber (control); SA = cucumbers with coating containing sodium lactate-acetic acid; SPA = cucumbers with coating containing sodium lactate-potassium sorbate-acetic acid; PA = cucumbers with coating containing potassium sorbate-acetic acid.

Values are mean \pm SD of two replicates

Values with different lowercase superscripts in a row are significantly different ($p \leq 0.05$)

Table 3 Sensory evaluation of uncoated and coated cucumbers stored for 3 days

Attribute	Coating formula			
	CON	SA	SPA	PA
Appearance	6.7 ± 0.1^a	6.8 ± 0.2^a	6.6 ± 0.1^a	6.5 ± 0.1^a
Color	6.7 ± 0.1^{ab}	6.8 ± 0.1^a	6.6 ± 0.1^b	6.7 ± 0.0^{ab}
Flavor	6.7 ± 0.5^a	6.8 ± 0.3^a	6.7 ± 0.5^a	6.5 ± 0.6^a
Texture	6.9 ± 0.2^a	6.7 ± 0.1^a	6.6 ± 0.2^a	6.4 ± 0.2^a
Overall liking	7.1 ± 0.4^a	6.9 ± 0.1^a	6.6 ± 0.3^a	6.7 ± 0.4^a

CON = uncoated cucumber (control); SA = cucumbers with coating containing sodium lactate-acetic acid; SPA = cucumbers with coating containing sodium lactate-potassium sorbate-acetic acid; PA = cucumbers with coating containing potassium sorbate-acetic acid.

Values are mean \pm SD of two replicates

Values with different lowercase superscripts in a row are significantly different ($p < 0.05$)

neutral ones. Regarding effects of both water solubility and low pH, the tapioca flour coating containing sodium lactate, potassium sorbate and acetic acid (SPA), provided the best inhibitory effect on the three pathogens (*E. coli*, *S. aureus*, and *Aspergillus* sp.). This was similar to the results reported by Vasconez et al. (2009), where the addition of potassium sorbate had no influence on water solubility. Recently, Saha et al. (2016) reported that starch (guar gum)-based edible coating was able to inhibit *S. aureus* and *E. coli* (food pathogenic bacteria) based on agar well assay, which was consistent with the current results. However, various antimicrobial agents in the coating matrix have different interactions; therefore, inhibition of selected agents should be considered (Chiu and Lai, 2010). The results of the study may have been mainly influenced by the synergistic effects of the preservative combinations and also the antibacterial effect of organic acids (Daskalov, 2012).

Application of the tapioca starch-organic salts-acetic acid coating on contaminated cucumbers was performed to evaluate the decontamination and shelf life of cucumber, since cucumbers typically contain 95% water, which is a favorite factor for the growth of bacteria and fungi (Patel and Panigrahi, 2019). The current work demonstrated that coating using tapioca starch incorporated with organic salts and acetic acid could inhibit microbial contamination on cucumbers. Coating of vegetables and fruits reduces cell wall degradation and prevents textural changes during storage (Charles et al., 2013); this would explain why the shelf life of cucumbers was improved. Moreover, tapioca starch coating can act as a semi-permeable membrane against diffusion of moisture, O₂, CO₂ and solute movements, leading to a reduced respiration rate (Raghav and Saini, 2018). The current found that the number of *E. coli* fell substantially after the application of the developed antimicrobial coating, indicating that all coating formulas could inhibit the growth of *E. coli*, which is sensitive to organic acids due to the ability of the dissociated acid to freely cross the cell membrane and release protons inside the cell (Hosein et al., 2011). Therefore, this improved the quality of the cucumbers inoculated with *E. coli* and their shelf-life improved, especially in the period of 24 h after the coating application. This finding was similar to that reported by Moreira et al. (2011) where the coating application of chitosan film was effective for endogenous *E. coli*. Shen et al. (2010) reported that bacterial differences were related to the sensitivity of *E. coli* and *S. aureus* to potassium sorbate, which could depress the internal pH in microbial cells and disrupt substrate transport by altering cell membrane permeability or reducing the proton motive force. Therefore, this developed coating had different effects on the tested pathogens on coated and uncoated cucumbers, whereas lower efficiency on *S. aureus* and *Aspergillus* sp. decontamination were observed. Generally, all coatings had similar effects on *Aspergillus* sp. inhibition. The possible explanation might be that *Aspergillus* sp. may more effectively resist the lethal effects of organic acids and salts compared to the other bacteria. In addition, the results of this study showed that the storage temperature and the surrounding relative humidity affected the shelf life of the coated and uncoated cucumbers. At refrigeration temperature (4°C), the antimicrobial activity of these three coating formulas effectively inhibited microbial

growth, leading to better quality during 12 d of storage compared to at ambient temperature (25°C). However, the organism may survive in the refrigerator; as many studies have reported it can survive even longer at refrigeration temperature (4°C) in acidified vegetables that have not been heated. Nevertheless, low temperature storage did result in a substantial reduction in the numbers of microbes on cucumbers in the current study. This result was similar to Moreira et al. (2015) who reported gellan gum edible coating applied on fresh-cut apple resulted in reductions in the mesophilic and psychrophilic counts.

Supporting the quality of cucumber was confirmed by sensory evaluation of five sensory attributes (appearance, color, flavor, texture and overall liking). Overall, the developed antimicrobial coating affected the appearance, color and texture of cucumbers, due to natural changes during storage. Similarly, Patel and Panigrahi (2019) reported that changes in the green skin color and other sensory quality deteriorated rapidly. The general findings in the current study indicated that the SPA coating provided better consumer acceptance after storage in contrast to the cucumbers coated with SA. However, the 3 d storage of coated cucumbers did not affect the scores for almost all attributes among cucumbers except for color change, whereas the coating solution contributed to a better sensory evaluation score perhaps because the addition of an organic salt, such as sodium lactate, in food at the concentrations used in the current study had no effect on appearance, texture and flavor. The physical and chemical properties of samples may have been slightly affected by the mixture of organic acids and salts, even though this salt can produce antioxidant activity (Bingol and Bostan, 2007), leading to appearance changes.

The developed antimicrobial coating of tapioca starch incorporated with sodium lactate, potassium sorbate and acetic acid (SPA) produced the best inhibition effect on *E. coli*, followed by *S. aureus* and *Aspergillus* sp. The mixture of organic acid and organic salts has been reported to be more efficient in pathogen inhibition (Daskalov, 2012). The antimicrobial migration in the coating matrix can have different functions, whereas only one antimicrobial agent showed no inhibitory effect. The results of surface coating application on cucumbers demonstrated that the antimicrobial treatments resulted in a significant bactericidal effect on *E. coli*, being effective in extending product shelf-life and inhibiting *E. coli* growth at the time of coating, while the coating slightly affected *S. aureus* and *Aspergillus* sp. during storage. In addition, the coating provided acceptable sensory quality and had little effect on overall acceptance by the panelists. In summary, the developed antimicrobial coating could improve the quality of cucumbers, especially against *E. coli*, at an affordable price, reducing economic loss due to spoilage. For other fruits and vegetables, the coating solution should be further developed considering various factors, such as food type, type of pathogen and storage conditions, before being applied on a specific product.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Bennett, R.W., Lancette, G.A. 1998. *Staphylococcus aureus*. In: Food and Drug Administration (Ed.). Bacteriological Analytical Manual, Revision, A, 8th ed. Association of Official Analytical Chemists. Washington, DC, USA, pp. 1–6.

Bingol, E.B., Bostan, K. 2007. Effect of sodium lactate on the microbiological quality and shelf life of sausages. *Turk. J. Vet. Anim. Sci.* 31: 333–339.

Campos, C.A., Gerschenson, L.N., Flores, S.K. 2011. Development of edible films and coatings with antimicrobial activity. *Food Bioprocess Tech.* 4: 849–875.

Charles, A., Kayode, A., Oluyemisi, F., Juliana, A., Olagbaju, A.R. 2013. Effect of edible coatings from carboxymethylcellulose and corn starch on cucumber stored at ambient temperature. *Asian J. Agri. Biol.* 1: 133–140.

Chiu, P.E., Lai, L.S. 2010. Antimicrobial activities of tapioca starch/decolorized hsian-tsao leaf gum coatings containing green tea extracts in fruit-based salads, romaine hearts and pork slices. *Int. J. Food Microbiol.* 139: 23–30. doi.org/10.1016/j.ijfoodmicro.2010.01.010

Dangaran, K., Tomasula, P.M., Qi, P. 2009. Structure and function of protein-based edible films and coatings. In: Embuscado, M.E., Huber, K.C. (Eds.). *Films and Coatings Edible Films and Coatings for Food Applications*. Springer-Verlag. New York, NY, USA, pp. 25–56.

Daskalov, H. 2012. Effect of different organic acid treatments with similar pH to control *Listeria monocytogenes* on cooked frankfurters. *Trakia J. Sci.* 10: 70–75.

Durango, A.M., Soares, N.F.F., Andrade, N.J. 2006. Microbiological evaluation of an edible antimicrobial coating on minimally processed carrots. *Food Control.* 17: 336–341. doi.org/10.1016/j.foodcont.2004.10.024

Feng, P., Weagant, S.D., Grant, M.A., Burkhardt, W. 2002. Enumeration of *Escherichia coli* and the Coliform Bacteria. In: Food and Drug Administration (FDA), Bacteriological Analytical Manual Online, 8th Ed., Silver Spring. Maryland, MD, USA. <http://www.fda.gov>, 23 May 2019

Flores, S., Fama, L., Rojas, A.M., Goyanes, S., Gerschenson, L. 2007. Physical properties of tapioca-starch edible films: Influence of filmmaking and potassium sorbate. *Food Res. Int.* 40: 257–265. doi.org/10.1016/j.foodres.2006.02.004

Hosein, A.M., Breidt, F., Smith, C.E. 2011. Modeling the effects of sodium chloride, acetic acid, and intracellular pH on survival of *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.* 77: 889–895. doi: 10.1128/AEM.02136-10.

International Standard ISO 7954 (ISO 7954). 1987. Microbiology - General Guidance for Enumeration of Yeasts and Molds - Colony Count Technique at 25 degrees celeus, International Organization for Standardization. Geneva, GE, Switzerland, pp. 6.

Kramer, M.E. 2009. Structure and function of starch-based edible. In: Embuscado, M.E., Huber, K.C. (Eds.). *Films and Coatings Edible Films and Coatings for Food Applications*. Springer. New York, NY, USA, pp. 113–134.

Mehyar, G., Al-Qadiri, H., Swanson, B. 2012. Edible coatings and retention of potassium sorbate on apples, tomatoes and cucumbers to improve antifungal activity during refrigerated storage. *J. Food Process. Pres.* 38: 175–182. doi.org/10.1111/j.1745-4549.2012.00762.x

Moreira, M.R., Cassani, L., Martín-Belloso, O., Soliva-Fortuny, R. 2015. Effects of polysaccharide-based edible coatings enriched with dietary fiber on quality attributes of fresh-cut apples. *J. Food Sci. Technol.* 52: 7795–7805. doi.org/10.1007/s13197-015-1907-z

Moreira, M., Roura, S., Ponce, A. 2011. Effectiveness of chitosan edible coatings to improve microbiological and sensory quality of fresh cut broccoli. *LWT-Food Sci. Technol.* 44: 2335–2341. doi.org/10.1016/j.lwt.2011.04.009

Patel, C., Panigrahi, J. 2019. Starch glucose coating-induced postharvest shelf-life extension of cucumber. *Food Chem.* 288: 208–214. doi.org/10.1016/j.foodchem.2019.02.123

Raghav, P.K., Saini, M. 2018. Development of mint (*Mentha viridis L.*) herbal edible coating for shelf life enhancement of cucumber (*Cucumis sativus*). *IJGHC.* 7: 379–391. doi: 10.24214/IJGHC/GC/7/2/37991

Reina, L.D., Fleming, H.P., Breidt, F. 2002. Bacterial contamination of cucumber fruit through adhesion. *J. Food Protect.* 65: 1881–1887.

Saha, A., Tyagi, S., Gupta, R.K., Tyagi, Y.K. 2016. Guar gum based edible coating on cucumber (*Cucumis sativus L.*). *Eur. J. Pharm. Med. Res.* 3: 558–570.

Shen, X.L., Wu, J.M., Chen, Y., Zhao, G. 2010. Antimicrobial and physical properties of sweet potato starch films incorporated with potassium sorbate or chitosan. *Food Hydrocolloid.* 24: 285–290. doi.org/10.1016/j.foodhyd.2009.10.003

Sonti, S. 2003. Consumer perception and application of edible coatings on fresh-cut fruits and vegetables. Bachelor thesis, The Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College, Osmania University College of Technology, Baton Rouge, LA, USA.

Taştan, Ö., Pataro, G., Donsi, F., Ferrari, G., Baysal, T. 2017. Decontamination of fresh-cut cucumber slices by a combination of a modified chitosan coating containing carvacrol nanoemulsions and pulsed light. *Int. J. Food Microbiol.* 260: 75–80. doi.org/10.1016/j.ijfoodmicro.2017.08.011

Valencia-Chamorro, S.A., Palou, L., Rio, M.A., Pérez-Gago, M.B. 2011. Antimicrobial edible films and coatings for fresh and minimally processed fruits and vegetables: A review. *Crit. Rev. Food Sci. Nutr.* 51: 872–900. doi.org/10.1080/10408398.2010.485705

Vasconez, M.B., Flores, S.K., Campos, C.A., Alvarado, J., Gerschenson, L.N. 2009. Antimicrobial activity and physical properties of chitosan-tapioca starch based edible films and coatings. *Food Res. Int.* 42: 762–769. doi.org/10.1016/j.foodres.2009.02.026