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Microbiological Evaluation of Edible Coated Fresh-Cut Cantaloupe

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ABSTRACT

This work aimed to investigate the effectiveness of the incorporation of chitosan as an antimicrobial additive in the edible methyl cellulose coating on the microbiological quality of fresh-cut cantaloupe. Cantaloupe slices were coated with edible coating solutions after washing in chlorinated water. The coating solutions were mixtures of 3% methyl cellulose and 2% glycerol that contained 0, 1, 1.5 and 2% of chitosan respectively. The slices were drained, packed on polystyrene trays and wrapped with polyvinylchloride film. The samples were stored at 10°C for 15 days. During storage, samples including a control (cantaloupe slices without coating) were collected every five days for microbiological analysis of mesophillic aerobes, psychrotrophs, lactic acid bacteria, yeast and mold, *Staphylococcus aureus*, total coliforms, *Escherichia coli* and *Salmonella* spp. Applications of 1.5 and 2% chitosan in the methyl cellulose coating of fresh-cut cantaloupe produced a better microbiological quality in the products. Chitosan reduced the growth of mesophillic aerobe (3.3 log cfu g^{-1}), psychrotrophs (3.9 log cfu g^{-1}), lactic acid bacteria (3.1 log cfu g^{-1}), yeasts and molds (1.1 log cfu g^{-1}) and total coliform (3.8 log cfu g^{-1}). Moreover, *S. aureus*, *Salmonella* sp. and *E. coli* were as low as <10 cfu g^{-1}, <10 cfu g^{-1} and 0 MPN g^{-1}, respectively.

Key words: fresh-cut, cantaloupe, edible coating, chitosan, microbiological quality

INTRODUCTION

Recently, there has been an increasing market demand for minimally processed fresh-cut fruit and vegetables due to their freshness convenience and human-heath benefits. Cantaloupe (*Cucumis melo* var. *Cantalupensis*) is one of the most popular edible fruits, but the market is limited due to its fast deterioration during storage that can reduce its shelf life. The product loses its firmness and develops an unpleasant odor due to the high respiration rate causing microbiological deterioration, so that consequently it requires effective control of microbial growth (Barry-Ryan *et a*l., 2000). Microbial propagation on the surface of product can be controlled by the application of a biopolymer-based edible coating (Cisneros *et al*., 1997).

Methylcellulose is one of the polysaccharides used in an edible coating. Cellulose is the principal structural component of plants and the most abundant source of complex carbohydrate. Methylcellulose is flexible and transparent and can act as a barrier to moisture and oxygen. Several studies have suggested the use of methyl cellulose as a suitable coating material for several products (Erol and Sibel, 2003). The usefulness of cellulose as a material

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for edible films may be extended by chemical modification. One cellulose derivative is methyl cellulose, which is water soluble and has good film properties film. Glycerol and polyethylene glycols have been shown to be the most effective plasticizers for methylcellulose. Maftoonazard and Ramaswamy (2004) reported that coating avocado with methylcellulose extended the shelf life of the product. Moreover, Ayranci and Tunc (2004) showed that coating apricot and green peppers with methylcellulose reduced the loss of water and vitamin C. Nevertheless, methylcellulose has no antimicrobial properties to reduce microbial deterioration. Incorporating antimicrobial additives in the coating with methyl cellulose was recommended.

An edible, antimicrobial coating has been shown to be an efficient method for controlling microbial contamination. The growth of both deteriorating and pathogenic microorganisms may be prevented by incorporating antimicrobial agents into edible films or coatings (Debeaufort *et al*., 1998). The antimicrobial agents most commonly used in edible coatings are: sorbic acid, propionic acid, potassium sorbate, benzoic acid, sodium benzoate and citric acid (Quintavalla and Vicini, 2002). Bacteriocins, such as nisin and pediocin (Sebti and Coma, 2002); enzymes, such as peroxidase and lysozyme (Padgett *et al*., 1998); and polysaccharides such as chitosan (Debeaufort *et al*., 1998) are also used as antimicrobial agents.

Chitosan is a hydrophilic polymer obtained industrially by hydrolyzing the aminoacetyl group of chitin using an alkaline treatment. It has widely been used in antimicrobial films and coatings because of its antimicrobial effect. Chitosan reacts with the strongly electronegative microbial surface leading to changes in permeability, metabolic disturbances and eventually death (Romanazzi *et al*., 2002). It has been demonstrated that lower molecular weight chitosans (less than 10kDa) have greater antimicrobial activity than those of native chitosans (Uchida, 1989). Coating strawberries and raspberries with 2.0% chitosan was almost as effective as the fungicide TBZ in preventing spoilage during storage at 13°C (Zhang and Quantick, 1998). In addition, loss of color, wilting and fungal infection in cucumber and bell pepper were all improved by coating with 1.5% chitosan (El Ghaouth, 1991). Therefore, this experiment was aimed to evaluate the effect of incorporating chitosan in to the edible coating on the microbiological properties of fresh-cut cantaloupe during storage at 10°C.

MATERIALS AND METHODS

Preparation of edible coating solution

The coating solutions were prepared using methyl cellulose and chitosan. Methyl cellulose (3% w/w) was dissolved in a water-ethyl alcohol mixture (3:1) at 75°C under magnetic stirring for 15 min. Ethyl alcohol was used to reduce the drying time and obtain a transparent and shiny methyl cellulose coating. Glycerol (2% w/w) was then added as a plasticizer and the solution was stirred for another 10 min under the same condition. After that, chitosan was added at the concentration of 0, 1, 1.5 and 2% (w/w), having been previously dissolved in 0.4% glacial acetic acid and agitated for one min to homogenize the suspensions.

Preparation of sample and coating application

Cantaloupes were washed, peeled and cut into slices (3×5 cm). The cantaloupe slices were washed using chlorinated water (150 ppm) for three min and drained for 10 min. The samples were then immersed in different coating solutions for three min and air flow dried for one hour. The control (uncoated sample) was submerged in sterile, distilled water under similar conditions. All the samples were placed on polystyrene trays, wrapped with polyvinylchloride film and stored at 10°C for 15 days.

Microbiological analysis

To evaluate the microbiological efficiency of the antimicrobial coating on the fresh-cut cantaloupes, microbiological analyses of mesophillic aerobes, psychrotrophs, lactic acid bacteria, yeasts and molds, *Staphylococcus aureus*, total coliform, *Escherichia coli* and *Salmonella* sp. were carried out on all samples during storage for 0, 5, 10 and 15 days at refrigerated temperature. The microbiological methods used were based on the Bacteriological Analytical Manual (BAM) (2001).

Statistical analysis

A randomized complete block design with three replications was used in this experiment. Difference between treatments was determined using Duncan's multiple range test.

RESULTS AND DISCUSSION

During storage, all the samples showed nearly complete or total absence of *S. aureus*, *Salmonella* sp. and *E. coli* with counting of <10 cfu g^{-1} for *S. aureus* and *Salmonella* sp. and 0 MPN g^{-1} for *E. coli*, respectively. All types of coatings affected the growth of mesophilic aerobes, psychrotrophs, yeast and mold, lactic acid bacteria and total coliforms. ANOVA analysis (Table 1) was carried out on the mesophilic aerobes, psychrotrophs, yeast and mold, lactic acid bacteria and total coliform counts in the fresh-cut cantaloupe, with four coatings during the 15 days of storage at 10°C. The number of coatings, time and coating-time interaction showed significant differences ($p<0.05$).

It was recognized that the higher concentration of chitosan in the edible coating material, promoted the higher effectiveness of the antimicrobial properties (Table 2). In comparison to the control (uncoated samples), 1.5 and 2% chitosan showed the highest reduction of approximately 3-4 logs in the growth of mesophilic aerobes, which was used as the indicator of food quality (Anonymous, 2001), and psychrotroph counts, most commonly associated with spoilage in refrigerated foods (Garg *et al*., 1990). The presence of chitosan in the edible coating material at 1.5 and 2% showed a greater effectiveness on the growth inhibition of mesophilic aerobes than washing with 2.5% hydrogen peroxide or 200 ppm chlorine (1.9 logs reduction) (Dike *et al*., 2005). Coma *et al*. (2002) also concluded that a chitosan coating had a bactericidal effect on *Listeria monocytogenes* and that such activity was probably due to the positive charges of chitosan, which interfered with the negatively charged residues of macromolecules on the *Listeria* cell surface, presumably by competing with calcium for electronegative sites on the membrane. Moreover, chitosan levels of 1.5 and 2% reduced the psychrotroph counts below the detectable level of lower than 100 cfu g^{-1} throughout the storage period. Chitosan at levels of 1.5 and 2% also reduced the number of yeast and mold counts to lower than 2.0 log cfu g^{-1}. This was probably due to the fungicidal action of chitosan that caused alterations in the function of the cellular membrane (Fang *et al*., 1994).

Table 1 Summary of variance analysis of mesophilic aerobes, psychrotrophs, yeasts and molds, lactic acid bacteria and total coliforms in fresh-cut cantaloupes submitted to four coatings during 15 days of storage at 10°C.

Factors of variance	Mesophilic aerobes	Psychrotrophs	Yeast and mold	Lactic acid bacteria	Total coliforms
Coating	13.4671*	27.0379*	3.6657*	45.5701*	12.5984*
Time	24.7475*	26.6131*	2.3570	55.9015*	8.2145*
Coating x Time	2.3742*	10.7603*	0.7750	10.6690*	2.6177*

*Significant at 5% probability ($p < 0.05$).

Lactic acid bacteria are mostly present in minimally processed fruit and vegetables. Marchetti *et al*. (1992) reported lactic acid bacteria counts as high as 10^8 cuf g^{-1} in carrot salad after seven day at 5°C. This was caused by fermentation. Lactic acid bacteria counts of fresh-cut cantaloupes are shown in Table 3. These results show that 1.5 and 2% chitosan levels inhibited the growth of lactic acid bacteria throughout the storage with the count lower than 100 cfu g^{-1}. The highest count was found in the uncoated sample with 0 and 1% chitosan 5.1 and 4.4 log cuf g^{-1}, respectively, at the end of storage.

Total coliform count is an indicator of

Table 2 Effect of coatings on the growth of mesophilic aerobes, psychrotrophs and yeast mold count in fresh-cut cantaloupe stored at 10°C for 15 days.

Microorganisms	Chitosan(%)	Storage time (days)			
		0	5	10	15
Mesophilic aerobes	Uncoated	2.7±0.6*	2.8±0.7	5.8±0.7	6.6±0.6
(log cfu/g)	0	<2.0±0.0	4.0±0.6	4.8±1.0	5.6±1.2
	1	<2.0±0.0	<2.0±0.0	4.7±1.3	4.8±0.4
	1.5	<2.0±0.0	<2.0±0.0	3.1±0.2	3.3±0.1
	2	<2.0±0.0	<2.0±0.0	2.2±0.3	2.7±0.5
Psychrotrophs	Uncoated	<2.0±0.0	<2.0±0.0	4.0±0.1	5.9±0.7
(log cfu/g)	0	<2.0±0.0	<2.0±0.0	2.3±0.5	3.2±1.2
	1	<2.0±0.0	<2.0±0.0	<2.0±0.0	2.7±1.1
	1.5	<2.0±0.0	<2.0±0.0	<2.0±0.0	<2.0±0.0
	2	<2.0±0.0	<2.0±0.0	<2.0±0.0	<2.0±0.0
Yeast and mold	Uncoated	<2.0±0.0	<2.0±0.0	2.5±0.7	3.1±1.1
(log cfu/g)	0	<2.0±0.0	<2.0±0.0	2.3±0.5	3.0±0.5
	1	<2.0±0.0	<2.0±0.0	<2.0±0.0	2.3±0.9
	1.5	<2.0±0.0	<2.0±0.0	<2.0±0.0	<2.0±0.0
	2	<2.0±0.0	<2.0±0.0	<2.0±0.0	<2.0±0.0

* mean ± standard deviation

Table 3 Effect of coatings on lactic acid bacteria and total coliform counts in fresh-cut cantaloupe stored at 10°C for 15 days.

Microorganisms	Chitosan(%)	Storage time (days)			
		0	5	10	15
Lactic acid bacteria	Uncoated	<2.0±0.0*	2.6±0.6	4.4±0.5	5.1±0.3
(log cfu/g)	0	<2.0±0.0	2.7±1.0	3.9±0.5	4.4±0.4
	1	<2.0±0.0	<2.0±0.0	3.5±0.1	3.8±0.7
	1.5	<2.0±0.0	<2.0±0.0	<2.0±0.0	<2.0±0.0
	2	<2.0±0.0	<2.0±0.0	<2.0±0.0	<2.0±0.0
Total coliforms	Uncoated	0.0±0.0	2.3±1.3	3.0±1.6	4.8±1.4
(log MPN/g)	0	0.0±0.0	0.0±0.0	2.0±0.9	2.3±0.8
	1	0.0±0.0	0.0±0.0	0.0±0.0	2.1±0.9
	1.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

* mean ± standard deviation

Table 4 Microbiological quality of fresh cut cantaloupe stored at 10°C for 15 days.

Treatment	log CFU/g				
	Mesophillic	Psychrotrophs	Yeast&Mold	LAB	Total coliform
Uncoated	6.6 ± 0.6^{c*}	5.9 ± 0.7^{b}	3.1 ± 1.1^{a}	5.1 ± 0.3^{c}	4.8 ± 1.4^{b}
0% chitosan	5.6 ± 1.4^{bc}	2.7 ± 1.2^{a}	3.0 ± 0.9^{a}	4.4 ± 0.4^{bc}	2.3 ± 0.8^{a}
1% chitosan	4.8 ± 0.4^{b}	3.2 ± 1.1^{a}	2.3 ± 0.5^{a}	3.8 ± 0.7^{b}	2.1 ± 0.9^{a}
1.5% chitosan	3.3 ± 0.1^{a}	2.0 ± 0.0^{a}	2.0 ± 0.0^{a}	2.0 ± 0.0^{a}	1.0 ± 0.0^{a}
2% chitosan	2.7 ± 0.5^{a}	2.0 ± 0.0^{a}	2.0 ± 0.0^{a}	2.0 ± 0.0^{a}	1.0 ± 0.0^{a}

***** The same letter indicates no significant difference at 95% confidential level

microorganisms, which indicate that food has been exposed to conditions that pose an increased risk that the food may be contaminated with a pathogen or held under conditions conductive to pathogen growth. No growth of total coliforms was found in the samples coated with 1.5 and 2% chitosan throughout the storage period. This indicated that at the proper concentration of chitosan, total coliforms were completely inhibited (Zheng and Zhu, 2003). Compared to the uncoated sample, these treatments showed a reduction of 3.8 logs.

The microbiological quality of fresh-cut cantaloupe coated with and without chitosan at the end of the storage period is shown in Table 4. Edible coating improved the microbiological quality of the product. Incorporation of 1.5 and 2% chitosan provided the best microbiological quality (the lowest count in all types of microorganisms); while 0 and 1% chitosan levels were not significantly different ($p>0.05$). Moreover, coating fresh-cut cantaloupe with methylcellulose containing 1.5 and 2% chitosan extended the shelf life of the product by up to 15 days at 10°C.

CONCLUSION

Application of chitosan as an antimicrobial substance in an edible coating consisting of methyl cellulose is a viable option for controlling the microorganisms present in fresh-cut cantaloupe, since the growth of mesophilic aerobes, psychrotrophs, yeasts and molds, lactic acid bacteria, total coliforms, *Staphylococcus aureus* and *Escherichia coli* were considerably inhibited by the application of 1.5 and 2% chitosan in the coating. The combination of these treatments as a barrier may offer potential for the shelf-life extension of fresh-cut cantaloupe by up to 15 days at 10°C.

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