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Effect of chitosan/methyl cellulose films on microbial and quality characteristics of fresh-cut cantaloupe and pineapple

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ABSTRACT

Two experimental films were applied on fresh-cut cantaloupe and pineapple and their effects on microbial control and fruit quality were investigated during storage at 10 °C. Three types of films were used in this study: a commercial stretch film, an experimental chitosan/methyl cellulose film, and a chitosan/methyl cellulose film incorporating vanillin (vanillin film) as a natural antimicrobial agent. Fresh-cut fruit without any film wrapping served as controls. Chitosan/methyl cellulose film and vanillin film provided an inhibitory effect against *Escherichia coli* on fresh-cut cantaloupe. The chitosan/methyl cellulose film rapidly reduced the number of *Saccharomyces cerevisiae* yeast inoculated on cantaloupe and pineapple. Vanillin film was more efficient than chitosan/methyl cellulose in reducing the number of yeast, which decreased by 4 logs in fresh-cut pineapple on day 6. Vanillin film increased the intensity of yellow color of pineapple. Pineapple removed from stretch film had higher respiration rates and ethanol contents than other treatments. Unsurprisingly, the stretch film maintained the moisture content in fruit better than other treatments. However, vanillin film reduced the ascorbic acid content in pineapple. At the end of storage, ascorbic acid in pineapple wrapped with vanillin film was only 10% of its original concentration.

1. Introduction

Chitosan is the second most abundant polysaccharide on earth and is inherently antimicrobial (Goldberg et al., 1990). Furthermore, it provides films with good mechanical and oxygen barrier properties (Caner et al., 1998; Chen et al., 1996). Chitosan, however, has poor tensile strength when wet. It is rigid and has poor elongation properties. Blending cellulose with chitosan can be expected to correct these weaknesses; film flexibility has been shown to increase with increasing methyl cellulose content (Garcia et al., 2004).

The growth of microorganisms on the cut surfaces is a main cause of food spoilage for fresh-cut produce. The application of antibacterial substances directly onto a food has some limitations because the active substances can be neutralized, evaporated or they may inadequately diffuse into the bulk of the food (Torres et al., 1985; Siragusa and Dickson, 1992). The incorporation of antimicrobial agents into packaging can create an environment inside the package that may delay or prevent the growth of microorganisms on the product's surface and, hence, lead to an extension of its shelf-life. Antimicrobial packaging has attracted much attention from the food industry because of the increase in consumer demand for minimally processed and preservative-free products. Reflecting this demand, preservative agents (preferably natural preservatives) must be applied at the lowest effective level possible (Cha and Chinnan, 2004). According to Brody et al. (2001), the antimicrobial effect of chitosan occurs when organisms are in direct contact with the active sites of chitosan. When antimicrobial agents are incorporated into film, they diffuse out of the film, thus improving its antimicrobial efficacy. Zivanovic et al. (2005) applied chitosan-oregano essential oil (EO) in comparison with chitosan films on inoculated bologna meat samples stored for 5 d at 10 °C. Pure chitosan films reduced *Listeria monocytogenes* by 2 logs, whereas the films with 1 and 2% oregano EO decreased the numbers of *L. monocytogenes* by 3.6 to 4 logs and *Escherichia coli* by 3 logs. Pranoto et al. (2005) incorporated garlic oil, potassium sorbate and nisin in chitosan films. The activity of the antimicrobial films was tested against the food pathogenic bacteria, *E. coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *L. monocytogenes*, and *Bacillus cereus*. They found that the pure chitosan film had no inhibitory effect. Incorporation of 100 μL of garlic oil/g, 100 mg potassium sorbate/g or nisin at 51,000 IU/g of chitosan had antimicrobial activity against *S. aureus*, *L. monocytogenes*, and *B. cereus*.

Many consumers have concerns over the addition of chemical additives to food, and this has driven the food industry

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Table 1
Numbers of *Escherichia coli* on inoculated cantaloupe (log cfu/piece) during storage at 10 °C

	No film	Stretch film	Chitosan/methyl cellulose film	Vanillin film
Day 0	5.18 ± 0.00 f	5.18 ± 0.00 f	5.18 ± 0.00 f	5.18 ± 0.00 f
Day 1	4.50 ± 0.23 e	5.45 ± 0.19 fg	3.88 ± 0.52 de	4.43 ± 0.20 e
Day 2	3.94 ± 0.01 de	5.95 ± 0.05 g	2.29 ± 0.83 c	3.54 ± 0.58 d
Day 4	2.86 ± 0.15 c	7.19 ± 0.22 h	1.00 ± 0.00 b	1.00 ± 0.00 b
Day 6	2.56 ± 0.15 c	8.98 ± 0.05 i	0.74 ± 0.00 b	0.74 ± 0.00 b
Day 8	1.00 ± 0.00 b	9.27 ± 0.05 i	0 a	0 a

Means with different letters are significantly different at $p = 0.05$.

and food research towards the search for natural antimicrobial compounds (Devlieghere et al., 2004). Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the major constituent of vanilla beans and is a flavor compound used in many baked or processed foods. Prindle and Wright (1977) found that the effect of phenolic compounds was concentration dependent. At low concentrations, phenols affected enzyme activity, especially those enzymes associated with energy production, while at greater concentrations, they caused proteins to denature. The antimicrobial activity of vanillin depended on the time of exposure, concentration and the target organism. Recent reports have shown that vanillin can be effective in inhibiting bacteria, yeasts and molds (Jay and Rivers, 1984; Cerrutti and Alzamora, 1996; Matamoros-Leon et al., 1999; Fitzgerald et al., 2004). Vanillin has been used to inhibit *E. coli O157:H7* and *L. monocytogenes* in 'Granny Smith' apple juice (Moon et al., 2006). Rupasinghe et al. (2006) reported that total aerobic counts of fresh-cut apple slices decreased from 4.3 log cfu/g fresh weight (untreated) to 1.6 log/cfu by using NatureSeal (an anti-browning agent) plus 12 mM vanillin after 19 d at 4 °C. Cerrutti et al. (1997) treated strawberry puree with a mild heat treatment combined with 3000 mg/L vanillin and 500 mg/L ascorbic acid. They found that the inhibition of native and inoculated flora growth for at least 60 d storage at room temperature. Penney et al. (2004) found that vanillin at 2000 mg/L suppressed fungal and total microbial growth in yoghurt significantly over the 3-week period.

Research on the application of antimicrobial biodegradable films on fresh-cut fruit is limited. The objectives of this work were to evaluate the inhibitory effect of chitosan/methyl cellulose film and chitosan/methyl cellulose film with vanillin against *E. coli* and *Saccharomyces cerevisiae*, and to determine the effect of these films on fresh-cut cantaloupe and pineapple quality.

2. Material and methods

2.1. Film preparation

Chitosan with a degree of deacetylation of 90% and purity >99.75% (Bannawach Bio-line Co. Ltd., Thailand) was prepared by dissolving 1.5 g of chitosan in 100 mL of 1% acetic acid solution. One half grams of methyl cellulose, 1.5 g, (M-043, BENECEL®) was dissolved in 50% ethanol. One gram of polyethylene glycol (PEG) 400 was used as a plasticizer. Solutions of chitosan and methyl cellulose were mixed in a beaker with a stir bar and heated to 72 °C. Stearic acid, 0.075 g was added to improve the water barrier properties of the film. Vanillin, 0.9 g (Sigma, St. Louis, USA) was incorporated after the temperature of the solution reached its melting point (83 °C). The film-forming solution was filtered through a cheese cloth to remove undissolved parts, homogenized with a homogenizer, degassed, cast onto glass plates, and dried at 40 °C for 42 h. Dried films were peeled off and conditioned at 25 ± 2 °C, 50 ± 5% RH for at least 48 h prior to use. Film thickness was measured with a gauge micrometer GT-313-A (Taiwan) with an accuracy of 0.01 mm.

2.2. Fruit preparation

Cantaloupe (*Cucumis melo*) and pineapple (*Ananas comosus)* fruit were purchased from a wholesale market in Chiang Mai province, Thailand. Total soluble solids were measured to indicate fruit maturity. Cantaloupe and pineapple used in this study had total soluble solids in the range of 7.0–8.2 and 17.0–19.4%, respectively. Whole fruit were washed with 500 mg/L chlorine solution. The blossom and stem ends were discarded. Cantaloupe and pineapple were sliced longitudinally into 12 wedges and 8 wedges, respectively using a sanitized sharp knife and cutting board. Then, the seeds or core, and peel were removed. All knives, cutting boards and other equipment which come into contact with the fruit were sanitized by immersion in 1000 mg/L chlorine solution for 30 min before cutting.

2.3. E. coli and S. cerevisiae inoculation and determination of E. coli and S. cerevisiae number through incubation

Cantaloupe and pineapple wedges were cut into 2.5 cm × 2.5 cm × 0.5 cm pieces. They were then inoculated with 20 μL of approximately 10^5 cfu/mL *E. coli* (TISTR 780) or *S. cerevisiae* (TISTR 5240) suspensions on the top surface of each piece (Zivanovic et al., 2005). Then, commercial stretch film, M wrap®, chitosan/methyl cellulose film and chitosan/methyl cellulose film with vanillin were wrapped around each piece. Wrapped fruit were placed on polystyrene trays and stored at 10 °C up to 20 d. Inoculated fruit without any wrapping served

Table 2
Numbers of *Saccharomyces cerevisiae* on inoculated cantaloupe (log cfu/piece) during storage at 10 °C

	No film	Stretch film	Chitosan/methyl cellulose film	Vanillin film
Day 0	5.36 ± 0.00 bc	5.36 ± 0.00 bc	5.36 ± 0.00 [bc]	5.36 ± 0.00 bc
Day 1	5.07 ± 0.06 bc	5.26 ± 0.04 bc	2.83 ± 0.95 a	4.81 ± 0.19 bc
Day 2	5.11 ± 0.03 bc	5.38 ± 0.04 bc	3.00 ± 0.00 a	4.85 ± 0.22 bc
Day 4	5.39 ± 0.16 bc	6.81 ± 0.10 de	2.26 ± 1.16 a	4.31 ± 0.12 bc
Day 8	7.07 ± 0.49 e	8.47 ± 0.23 fg	4.24 ± 1.29 b	4.85 ± 0.11 bc
Day 12	6.72 ± 0.85 de	9.03 ± 0.11 gh	5.65 ± 0.58 bcd	4.45 ± 0.11 bc
Day 16	7.45 ± 0.38 ef	8.87 ± 0.14 gh	5.68 ± 0.55 cd	4.75 ± 0.05 bc
Day 20	7.27 ± 0.63 e	9.72 ± 0.16 h	5.20 ± 0.62 bc	4.71 ± 0.12 bc

Means with different letters are significantly different at $p = 0.05$.