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Effect of High Vanillin Treatment on Storage Quality of Fresh-cut Apples

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Abstract The effect of post-cut vanillin treatment at high concentrations on changes of quality and microorganism in fresh-cut apples was studied. Apples (*Malus domestica* Borkh. cv. Fuji) were sliced, treated by dipping in different vanillin solution, 0, 40, 80, and 120 mM, packed in polyethylene bag, and then stored for up to 3 weeks at 4°C. Changes in total aerobic bacteria, yeast and molds, browning, soluble solids, and titratable acidity during storage were investigated. Growth of total aerobic bacteria throughout storage was strongly inhibited by vanillin regardless of treatment concentrations. Growth of yeast and molds was inhibited by vanillin of all concentrations until 2 weeks of storage. Levels of browning index, soluble solids, and titratable acidity were not significant difference among the treatment conditions until 2 weeks of storage. However, when stored for 3 weeks, browning index increased more at 80 or 120 mM vanillin, while soluble solids and titratable acidity more be decreased by 120 mM vanillin as compared with other treatment conditions. These results show that the usage of vanillin in processing of fresh-cut apples had a limitation for maintaining quality.

Keywords: *Malus domestica* Borkh., fresh-cut, vanillin, antimicrobials, browning

Introduction

The industry of fresh-cut fruits and vegetables is continuously growing due to consumers demand for fresh, convenient, and nutritious foods (1). Fresh-cut products generally have a short shelf-life due to unique post-cut problems such as rapid loss of quality caused by cut-surface browning, tissue softening, physiological breakdown, and microbial growth (2,3). New techniques for maintaining quality and inhibiting undesirable microbial growth are demanded in all the steps of the production and distribution chain (1,4). In case of fresh-cut apples, the enzymatic browning is effectively restrained by the chemical agents such as ascorbic acid, citric acid, cysteine, and other compounds derived from natural resources, and together with oxygen barrier coating and packaging (5-10). The softening is reduced with calcium salts and 1-methylcyclopropene treatments (11-13), and the change in nutritional components is controlled with a modified atmosphere packaging having various functions (14). The microbial growth in fresh-cut apples can be inhibited with a combination of antimicrobial agents and the use of modified atmosphere packaging (15,16). However, the inhibition of microbial growth for long-term storage has limitations due to a limited kind and usage of the agents for use in foods (17). Recently, the interest of using natural antimicrobial agents to suppress microbial growth on produce is the focus of researchers and consumers (15). As naturally occurring antimicrobials, vanillin, benzaldehyde, ferulic acid, estragole, guaiacol, and eugenol were well known (18).

Vanillin (4-hydroxy-3-methoxybenzaldehyde), a predominant phytochemical that occurs in vanilla beans and generally regarded as safe (GRAS) flavoring agents used widely in food processing (19), is an antioxidant (20,21), antimutagenic, and antigenotoxic compounds (22,23), and is also known to be antimycotic and bacteriostatic compounds (24,25). It has been reported that 12 or 10-20 mM vanillin are effective in inhibiting food spoilage yeasts in apple puree (26) or some yeasts in apple juice (27), and 3-7 mM vanillin, or 3 mM vanillin combined with 2 mM potassium sorbate can inhibit the growth of some *Aspergillus* species in fruit-based agars (28), or *Penicillium* species in potato dextrose agar (29). It was also reported that vanillin (6-18 mM) has antimicrobial effect against *Candida albicans, Enterobacter aerogenes, Escherichia coli*, *Lactobacillus casei*, *Penicillum expansum*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae,* and *Salmonella enterica* in culture media. In addition, it was observed that 12 mM vanillin do not have an influence on enzymatic browning and softening by calcium ascorbate in fresh-cut apples (30). However, the effects of a higher concentration of vanillin on the microbial growth as well as the change of quality characteristics in fresh-cut apples are not yet reported.

The purpose of this work was to study the application possibility of high level vanillin as natural antimicrobial agents and healthy materials in fresh-cut processing of apples; we determined the pretreatment effect of 40-120 mM of vanillin on the growths of total aerobic bacteria, yeast and molds, the changes of surface color, soluble solids, and titratable acidity in fresh-cut apples during storage.

Materials and Methods

Materials Apples (*Malus domestica* Borkh. cv. Fuji) were harvested at commercial maturity from the orchard of the

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Pacific Agri-Food Research Centre at Summerland, BC, Canada on 2005 and graded according to size and defects, were immediately placed in a cold room. Apples were stored for 1 month at 0°C, and used for the preparation of apple slices. The initial soluble solids and titratable acidity of the prepared apple slices were 15.61% and 0.39 mg/mL, respectively. Vanillin was obtained from Sigma-Aldrich (St. Louis, MO, USA). Petrifilm™AC count plate and Petrifilm™YM count plate were obtained from 3 M Microbiology Products (St. Paul, MN, USA). Other chemicals used for analyses were technical grade.

Preparation of apples slices Whole apples were washed in the 100 μL/L sodium hypochlorite solution (pH 6.5, 5°C) for 2 min and dried for 10 min, then cored and sliced into 8 equal wedges with skin using a sharp stainless steel knife. After slicing, the slices were dipped in an aqueous solution of 0, 40, 80, or 120 mM vanillin for 3 min, and then were allowed to drain. Six slices were randomly selected and packed in 0.04-mm low density polyethylene bags. The bagged apple slices were kept at 4°C and removed on 1, 2, and 3 week intervals for testing colony counts of microorganisms and analyzing color, soluble solids, and titratable acidity. The experiment was conducted in triplicate for each vanillin concentration.

Total aerobic bacteria, yeast, and molds analysis Twenty-five g of apple slices were homogenized with 225 mL of 0.1% peptone for 1 min. Aliquots (1 mL) were plated in duplicate onto Petrifilm™ AC and incubated at 37°C for 48 hr for total aerobic bacteria analysis. Aliquots (1 mL) were inoculated onto Petrifilm™ PM and incubated at 25°C for 5 days for yeast and molds analysis. Petrifilm™ having counts ranging from 20 to 200 CFU were considered to be within the countable range (30).

Color analysis Color of the cut surface was determined using a colorimeter (CR-300; Minolta Co., Osaka, Japan) calibrated with a standard white plate (X=0.93, Y=0.95, Z=1.09). Three reading of X, Y, and Z were recorded for each slice around the mid point area between endocarp and skin. Numerical values of X, Y, and Z were converted into browning index according to the following equation (9).

$$\text{Browning index}=(x-0.31)\times 100/0.172$$

where, x is the chromaticity coordinate calculated from the X, Y, and Z values according to the following equation $x = X/(X+Y+Z)$.

Soluble solids analysis Apple slices were homogenized and filtered. Content of soluble solids of the filtrate was measured with a digital refractometer (PR-101; Atago Co., Tokyo, Japan).

Titratable acidity analysis Ten g of apples slices were homogenized in 90 mL of distilled water and filtered. Titratable acidity of the filtrate was measured by titrating 20 mL of juice with 0.1 N NaOH to pH 8.2 and expressed as mg equivalent malic acid/mL juice.

Statistical analysis The data were subjected to analysis of variance (AVOVA) using the general linear model (GLM) procedure of the SAS statistical package (Statistical Analysis System, SAS Institute, Cary, NC, USA). Mean separation was determined using Duncan's multiple-range test ($p<0.05$).

Results and Discussion

Effect of vanillin on microbial growth The changes in the growth of total aerobic bacteria in fresh-cut apples as affected by concentrations of vanillin treatment during storage at 4°C are presented in Fig. 1. The growth of total aerobic bacteria was inhibited by vanillin at 40, 80, and 120 mM concentrations over 3 weeks storage period. Anti-aerobic microbial effect had no significant differences among the vanillin concentrations at each storage period. Colony count of total aerobic bacteria of vanillin non-treated apple slices was increased as storage period increased, while those of vanillin treated apple slices showed no changes until 2 weeks of storage and then slightly increased after 3 weeks of storage. Otherwise, Rupasinghe *et al.* (30) reported that the growth of pathogenic, indicator, and spoilage organisms in fresh-cut apples display an inverse relationship with increase of vanillin concentration up to 18 mM. In addition, the minimal inhibitory concentration of vanillin between 6 and 18 mM is dependent upon the microorganism. In this study, we found that the efficacy of high levels (≥40 mM) of vanillin on microorganism reduction was limited.

The changes of yeast and molds in fresh-cut apples in relation to concentrations of vanillin post-cut treatment throughout storage at 4°C are shown in Fig. 2. In general, the colony count of yeast and molds in fresh-cut apples during storage increased with increasing storage periods. The growth of yeast and molds was inhibited by vanillin post-cut treatment until 2 weeks of storage. Among fresh-cut apples treated with vanillin, the colony count of yeast and molds was lower in fresh-cut apples treated with 80 or 120 mM vanillin than in those treated with 40 mM vanillin. However, there was no significant difference between vanillin treated and vanillin non-treated fresh-cut apples after 3 weeks of storage. Cerrutti and Alzamora (26) reported that 12 mM vanillin inhibits the growth of *Debaryomyces hansenni*, *S. cerevisiae*, *Zygosaccharomyces rouxii*, and *Zygosaccharomyces bailii* in apple puree. Fitzgerald *et al.* (27) found that *S. cerevisiae* and *Candida parapsilosis* in apple juice are inhibited by 10 and 20 mM vanillin, while Lopez-Malo *et al.* (28) reported that 3-7 mM vanillin inhibit the growth of some *Aspergillus* species in fruit-based agars. Furthermore, Matamoros-Leon *et al.* (29) suggested that 3 mM vanillin combined with 2 mM potassium sorbate inhibit the growth of *Penicillium digitatum*, *Penicillium glabrum*, and *Penicillium italicum* in potato dextrose agar. From the above results, we indicated that the inhibitory effect of vanillin against yeast and molds in fresh-cut apples strongly depended on post-cut storage period and higher level of vanillin.

Effect of vanillin on quality change The browning of the cut-surface is the major obstacle in processing and marketing of fresh-cut apples. The browning is undesirable not only because of discoloration of the product but also in relation to the associated reactions that produce off-flavors. In this study, the degree of browning was represented as a

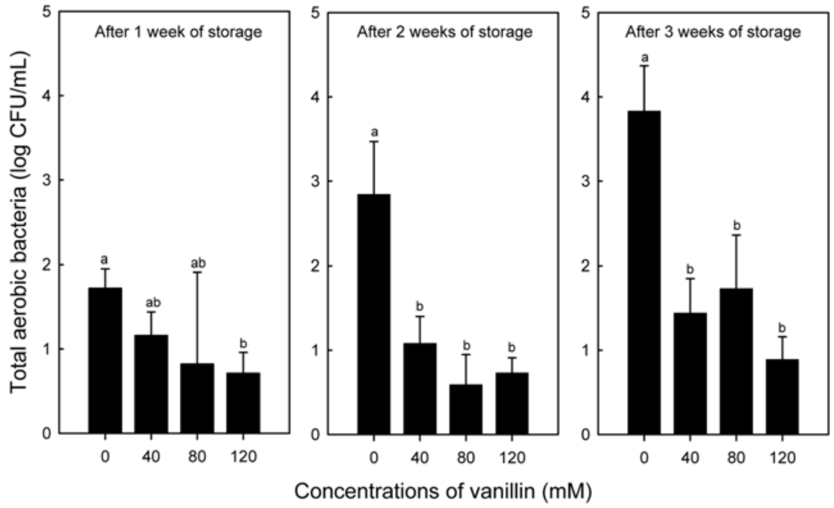

Fig. 1. Effect of vanillin concentration on the total aerobic bacteria of fresh-cut apple slices during storage at 4°C. Value are mean±SD (n=3), means with the different upper letter were significantly different (p<0.05).

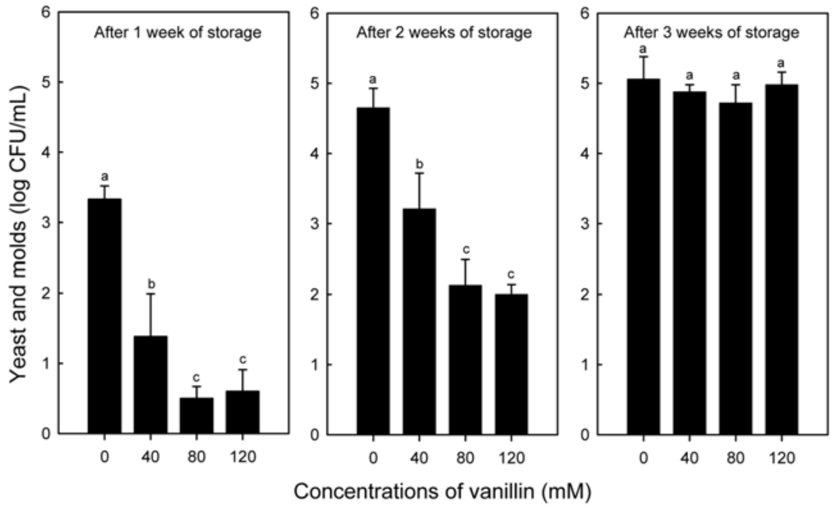

Fig. 2. Effect of vanillin concentration on the yeast and molds of fresh-cut apple slices during storage at 4°C. Value are mean±SD (n=3), mean with the different upper case letter were significantly different (p<0.05).

browning index calculated from X, Y, and Z value. The changes in browning index of post-cut vanillin treated or non-treated apple slices during storage are shown in Fig. 3. The browning index tended to increase with increasing storage periods. There was no significant difference among the treatment conditions until 2 weeks of storage. However, the browning index in fresh-cut apples treated with 80 or 120 mM vanillin were significantly higher than that in fresh-cut apples treated with 0 or 40 mM vanillin after 3 weeks of storage. It is well known that the browning reaction in fresh-cut apples is mainly induced by polyphenol oxidase (31). These results demonstrate that vanillin below 40 mM did not have an effect on the enzymatic browning reaction in fresh-cut apples, but above 80 mM vanillin accelerates the browning in fresh-cut apples during prolonged storage periods. Previous study (30) had shown that flesh color of 'Empire' and 'Crispin' apple slices was unaffected by 12 mM vanillin combined with a calcium ascorbate.

The sugar and acid contents greatly affect the sensory quality of fruits. The main sugars in apples are fructose, sucrose, and glucose, and the main acid is malic acid (32). Soluble solids content is an approximate measurement of the sugar content of a fruit. The changes of soluble solids in fresh-cut apples during storage at 4°C are presented in Fig. 4. Soluble solid contents of all apple slices except the slices treated with 120 mM vanillin did not vary from the initial contents, and was unaffected by varying the concentrations of vanillin. After 3 weeks of storage, the soluble solids contents of apple slices treated with 120 mM vanillin were significantly lower than those of other apple slices. This result suggests that post-cut treatment of 120 mM vanillin can be decreased the soluble solids of fresh-cut apples during prolonged storage.

Titratable acidity is a quantitative measure of the organic acids which are present in a fruit. The changes of titratable acidity in fresh-cut apples in relation to the concentration of vanillin treatments during storage are shown in Fig. 5.

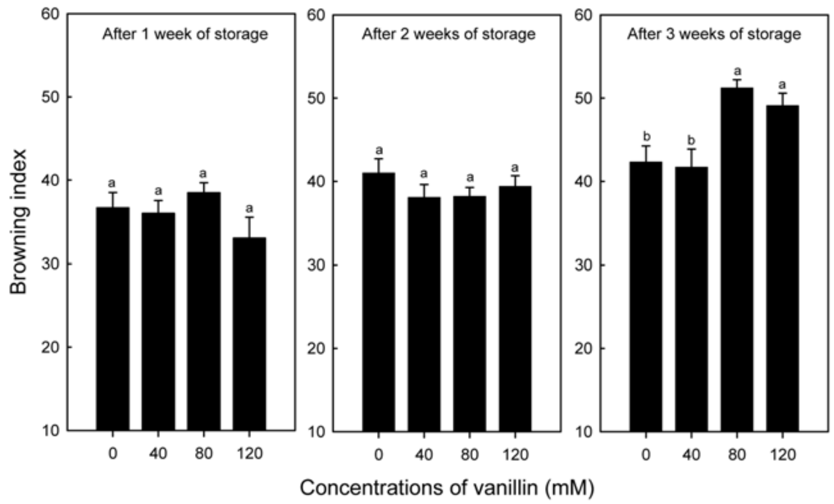

Fig. 3. Effect of vanillin concentration on the browning index of fresh-cut apple slices during storage at 4°C. Value are mean±SD (n=3), mean with the different upper case letter were significantly different (p<0.05).

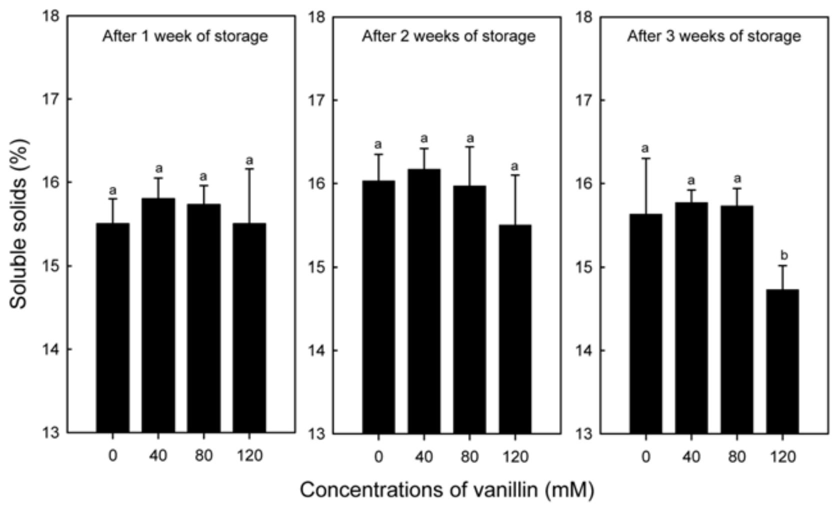

Fig. 4. Effect of vanillin concentration on the soluble solids contents of fresh-cut apple slices during storage at 4°C. Value are mean±SD (n=3), mean with the different upper case letter were significantly different (p<0.05).

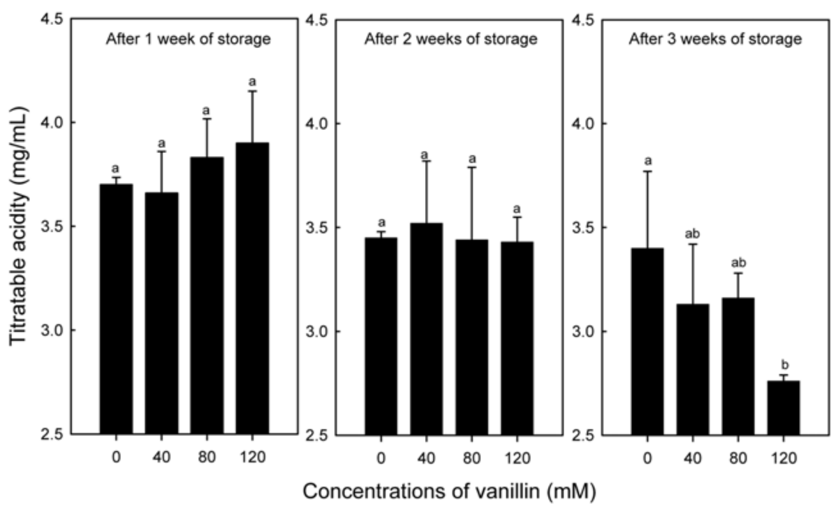

Fig. 5. Effect of vanillin concentration on the titratable acidity of fresh-cut apple slices during storage at 4°C. Value are mean±SD (n=3), mean with the different upper case letter were significantly different (p<0.05).

Titratable acidity in all fresh-cut apples tended to decrease with increasing storage periods. This decrease was attributed to the increased in respiration after cutting, given that organic acids are known to be used quickly by reactions involved in respiration (33). The loss rate of titratable acidity in apple slices treated with vanillin was not significantly different from non-treated apple slices until 2 weeks of storage. However, titratable acidity of apple slices treated with vanillin was lower than in non-treated apple slices after 3 weeks of storage. Among apple slices treated with vanillin, those treated with 120 mM had the lowest titratable acidity. From the above results, we suggest that high vanillin treatment had promotion effective of titratable acidity loss in fresh-cut apples during long-term storage.

In conclusion, vanillin was found to be effective in inhibiting growth of total aerobic bacteria, yeast and molds in fresh-cut apples. However, white color, soluble solids, and titratable acidity in apple slices could be decreased by high vanillin concentrations. Therefore, this study provides that the usage of vanillin in processing of fresh-cut apples has a limitation for maintaining quality. Further research is required in order to obtain information about the possible mechanism of action of high vanillin against decreasing of quality characteristics.

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