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Vanillin inhibits pathogenic and spoilage microorganisms in vitro and aerobic microbial growth in fresh-cut apples ☆

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Abstract

The antimicrobial effect of vanillin against four pathogenic or indicator organisms; *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, and *Salmonella enterica* subsp. *enterica* serovar Newport and four spoilage organisms; *Candida albicans*, *Lactobacillus casei*, *Penicillum expansum*, and *Saccharomyces cerevisiae* that could be associated with contaminated fresh-cut produce, was examined. The minimal inhibitory concentration (MIC) of vanillin was dependent upon the microorganism and this ranged between 6 and 18 mM. When incorporated with a commercial anti-browning dipping solution (calcium ascorbate, NatureSeal™), 12 mM vanillin inhibited the total aerobic microbial growth by 37% and 66% in fresh-cut 'Empire' and 'Crispin' apples, respectively, during storage at 4 °C for 19 days. Vanillin (12 mM) did not influence the control of enzymatic browning and softening by NatureSeal. These results provide a new insight for vanillin as a potential antimicrobial agent for refrigerated fresh-cut fruits and vegetables.

Keywords: Vanillin; Natural antimicrobial; MIC; Fresh-cut apple; Shelf life; Pathogenic and spoilage microorganisms

1. Introduction

There has been a steady increase in consumer demand for convenient and nutritious minimally processed produce like fresh-cut apples (Gorny, 2003). Current fresh-cut processing technologies such as post-cut dipping in calcium ascorbate (NatureSeal™) to prevent enzymatic browning and softening allows a shelf life of up to 21 days for sliced apples (Chen, 1999; Rupasinghe, Murr, DeEll, & Odumeru, 2005). However, the fresh-cut produce industry is challenged with potential outbreaks of illness that could be associated with microbial growth during the extended shelf life of these products (Alzamora & Guerrero, 2003). These current trends in the fresh-cut apple industry have led to a growing interest in investigating natural antimicrobial agents that are compatible with the chemical properties of post-cut dipping solutions of fresh-cut apples (Alzamora & Guerrero, 2003).

Naturally occurring antimicrobials include compounds derived from biological materials (Brul & Coote, 1999). There are three classes of naturally occurring antimicrobial substances: (1) animal-derived enzymes (lysozyme, lactoperoxidase), other proteins (lactoferrin, lactoferricin, and ovotransferrin), and small peptides (histatins and magainins); (2) plant-derived secondary metabolites (phytoalexins, phenolics, and essential oils); and (3) microorganism-based bacteriocins (nisin and pediocin) (Beuchat & Golden, 1989; Brul & Coote, 1999; Gould, 1996). Recently, many plant extracts have been shown to possess antimicrobial activities against a wide range of microorganisms related to food spoilage and safety (Friedman, Henika, & Mandrell, 2002; Patrzykat & Douglas, 2003). Little is

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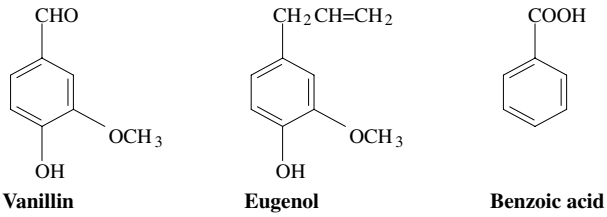
known about the resistance mechanisms of microorganisms against these naturally occurring antimicrobial compounds, although many, such as vanillin, benzaldehyde, ferulic acid, estragole, guaiacol and eugenol, are hydrophobic and contain aromatic structures similar to the ones found in classical preservatives such as benzoic acid (Fig. 1) (Brul & Coote, 1999; Cerrutti & Alzamora, 1996).

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the predominant phytochemical that occurs in vanilla beans and is a generally regarded as safe (GRAS) flavoring compound used widely in ice cream, beverages, biscuits, chocolate, confectionary, desserts, etc. (Beuchat & Golden, 1989; Hocking, 1997; Ramachandra Roa & Ravishankar, 2000). Vanillin, present in the essential oil fraction of the vanilla bean, is structurally similar to eugenol (2-methoxy-4-(2-propenyl)phenol) from cloves and is known to be antimycotic (Beuchat & Golden, 1989) and bacteriostatic (Fitzgerald et al., 2004a). Inhibitory action of vanillin at MIC was found to be bacteriostatic in contrast to the more potent phenolic antimicrobials such as carvacrol and thymol (Fitzgerald et al., 2004a) that are bactericidal (Friedman et al., 2002; Ultee, Bennik, & Moezelaar, 2002). Based on the studies conducted using *Escherichia coli*, *Lactobacillus plantarum*, and *Listeria innocua*, the inhibitory activity of vanillin resides primarily in its ability to detrimentally affect the integrity of the cytoplasmic membrane, with the resultant loss of ion gradient, pH homeostasis and inhibition of respiratory activity (Fitzgerald et al., 2004a).

Recently, it has been shown extensively that vanillin is effective in inhibiting yeast and moulds in vitro (Cerrutti & Alzamora, 1996; Fitzgerald, Stratford, & Narbad, 2003; López-Malo, Alzamora, & Argaiz, 1995, 1997, 1998; Matamoros-León, Argaiz, & López-Malo, 1999) and in fruit puree or juice (Castañón, Argaiz, & López-Malo, 1999; Cerrutti & Alzamora, 1996; Cerrutti, Alzamora, & Vindales, 1997; Fitzgerald, Stratford, Gasson, & Narbad, 2004b). Vanillin (12 mM) inhibited the growth of four food spoilage yeasts, *Saccharomyces cerevisiae, Zygosaccharomyces rouxii, Debaryomyces hansenni,* and *Zygosaccharomyces bailii*, in culture media and apple puree for 40 days storage at 27 °C (Cerrutti & Alzamora, 1996). Incorporation of vanillin (3–7 mM) into fruit-based agars inhibited the growth of four *Aspergillus* species for 2 months (López-Malo et al., 1995). When combined with 2 mM potassium sorbate, 3 mM vanillin could inhibit the growth of three



Vanillin **Eugenol** **Benzoic acid**

Fig. 1. The chemical structure of vanillin in comparison to eugenol and commercial preservative benzoic acid.

Penicillium species: *Penicillium digitatum*, *Penicillium glabrum*, and *Penicillium italicum*, grown in potato dextrose agar (pH 3.5, a_w 0.98) for 1 month (Matamoros-León et al., 1999). Fitzgerald et al. (2004b) reported that two yeast strains, *S. cerevisiae* and *Candida parapsilosis*, inoculated at a level of $\sim 10^4$ cfu/ml in apple juice and peach-flavored soft drink were inhibited by vanillin at 20 and 10 mM concentrations, respectively, over an 8-week storage period at 25 °C.

Therefore, an investigation of antimicrobial properties of vanillin when incorporated in calcium ascorbate (NatureSeal™), a commercial post-cut dipping solution, could offer new opportunities for extending the shelf life of fresh-cut fruits. The objectives of this study were to determine: (i) dose-dependent effectiveness of vanillin as a natural antimicrobial agent against selected pathogenic, indicator, and spoilage organisms and (ii) effectiveness of vanillin (12 mM) when incorporated with NatureSeal to suppress the total aerobic microbial growth on fresh-cut 'Empire' and 'Crispin' apples.

2. Materials and methods

2.1. Microorganisms and culture conditions

All microorganisms used in this study were obtained from the American Type Culture Collection (ATCC), Manassas, VA. The pathogenic and indicator strains include: *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter aerogenes* (ATCC 13048), *Salmonella enterica* subsp. *enterica* serovar Newport (ATCC 6962), and the four spoilage organisms were *Candida albicans* (ATCC 10231), *S. cerevisiae* (ATCC 9763), *Penicillum expansum* (ATCC 7861), *Lactobacillus casei* (ATCC 7469). For the two yeast species, malt extract broth (MEB) growth medium was used, while Man Rogosa and Sharpe (MRS) agar was used for *L. casei*, and Trypticase soy broth (TSB) was used for the remaining cultures. These culture media were purchased from Oxoid Inc., Nepean, Ont., Canada.

2.2. Preparation of vanillin

Different vanillin concentrations (0, 1.5, 3, 6, 12, and 18 mM) were prepared in triplicate by adding the appropriate amounts of vanillin (Ashland Canada Inc., Mississauga, Ont., Canada) into flasks containing corresponding sterile growth medium and heating for 2 min using a microwave. The medium was then dispensed into 10 ml aliquots in sterile glass tubes.

2.3. Susceptibility testing

Inoculum for each of the microorganisms was prepared to a concentration of approximately 1×10^8 cfu/ml using McFarland standards (Med-Ox Diagnostics Inc., Ottawa, Ont., Canada) and 100 µl of inoculum was used to inoculate each of the prepared tubes containing culture media with