REVIEW ARTICLE

Natural antimicrobial agents of cinnamon (*Cinnamomum zeylanicum* L. and *C. cassia*) and vanilla (*Vanilla planifola, V. pompona, and V. tahitensis*) for extending the shelf-life of fresh-cut fruits

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ABSTRACT

Currently there is an increasing demand for fresh-cut fruits due to their convenience and health benefits. However, the different food borne disease outbreaks associated with fresh produce has challenged the fresh-cut fruit industry. Owing to the increasing consumers' concern about chemical preservatives and the stringent legislation on some of the existing preservatives, the need of substituting chemical preservatives with more consumer friendly and safe natural products is getting a growing demand. Many researchers have investigated the antimicrobial effects of spices and herbs such as cinnamon and vanilla, which have acceptable aroma and flavour for consumers in the North America. This review article describes the application of natural plant-derived antimicrobial agents in controlling pathogenic and spoilage microorganisms associated with fresh-cut fruits with special emphasis on the use of vanilla and cinnamon.

Keywords: Cinnamon, Fresh-cut fruits, Natural antimicrobials, Pathogenic and spoilage microorganism, Vanilla

INTRODUCTION

Fresh-cut products are defined as fruits or vegetables that have been trimmed and/or peeled and/or cut in to 100% usable product that is bagged or pre-packaged to offer consumers high nutrition, convenience and flavour while still maintaining freshness (Kader and Lamikanra, 2002; FDA, 2008). Fresh-cut fruit and vegetable consumption is growing rapidly and accounts for more than 10% of all produce in the United States with annual growth rate of 10% to 20% (Saftner *et al*., 2005; FDA, 2008). This increasing trend is typically due to the increasing demand of low caloric food product, which is natural and healthy, and most importantly there are much scientific evidence that consumption of fruits and vegetables help to prevent cardiovascular disease and several types of cancers (Rico *et al*., 2007).

Fruits and vegetables are commonly considered to have less microbial hazards as compared to animal products (Wiley, 1994). However, numerous food borne illness outbreaks related with fresh-cut fruits and vegetables have been reported in some countries. According to FDA (2008), fresh-cut produce accounted for 25% of food borne illnesses. A typical example was a Salmonella outbreak in cut melon. The outbreak was in USA and Canada in 1991, where about 450 cases were reported (Conway *et al*., 2000; Harris, *et al*., 2003).

Fresh-cut fruits are more perishable than the intact fruits. The reasons include the post-harvest damage during processing and handling, high moisture and nutrient content, absence of heat or other treatments to eliminate pathogenic and spoilage microbes and the possible temperature abuse during processing, storage and marketing of these products (Brecht, 1995; Heard, 2002). Combinations of different methods have been used to improve the microbial safety and quality of fresh-cut fruits. These include chilling, freezing, modified atmosphere packaging (MAP), and chemical preservatives (Raybaudi-Massilia *et al*., 2009). Due to the increasing consumers' concern about chemical antimicrobial agents and the strict regulation on the existing preservatives, the importance of replacing chemical preservatives with natural and accepted product such as extracts of fruits, spices and herbs have been investigated recently (Nychas *et al*., 2003; Muthuswamy and Rupasinghe, 2007). Currently, essential oils from different spices are found to have an outstanding effect against different microbes (Burt, 2004; Raybaudi-Massilia *et al*., 2009).

Cinnamon and vanilla are some of the popular and well-accepted flavours in the North America. They are incorporated in many foods such as ice cream, beverages, biscuits, chocolate and other products (Raybaudi-Massilia *et al*., 2008b). Their antimicrobial property has been known since antiquity and currently many studies have confirmed their effect against different bacteria, yeasts and moulds (Bullerman 1974**;** Roller and Seedhar, 2002; Raybaudi-Massilia *et al*., 2006; Rojas-Graü *et al*., 2007; Muthuswamy *et al*., 2008; Raybaudi-Massilia *et al*., 2008a; Raybaudi-Massilia, *et al*., 2008b; AL-Saghir *et al*., 2009).

This paper presents a brief review of the use of plant-derived natural antimicrobial agents in fresh-cut fruits with special focus on the use of vanilla and cinnamon in controlling pathogenic and spoilage microorganism of fresh-cut fruits. The paper will cover a brief explanation and discussion about 1) the major spoilage and pathogenic microbes in fresh-cut fruits, 2) synthetic vs. natural antimicrobial agents, 3) mode of action of the major antimicrobial agents in vanilla and cinnamon, and 4) the use of cinnamon and vanilla as antimicrobial agents on different fresh-cut fruits.

SPOILAGE AND PATHOGENIC MICROBES OF FRESH-CUT FRUITS

Microbial contamination of fresh-cut fruits is mostly originated from the soil, inadequately composted manure, contaminated water, insects, animals, and cross-contamination during post-harvest handling practices (Heard, 2002). Due to higher carbohydrates, low pH (<4.6) and low protein content of fruits, the common spoilage microbes are yeasts, moulds and acid resistant bacteria (Heard, 2002). Yeasts can cause more spoilage than moulds since they can multiply faster (Heard, 2002). Tournas *et al*. (2006) have studied the microbial load of 38 fruit salads including cantaloupe, citrus, pineapple, strawberries and mixed fruit salads, and found 97% contamination with yeasts such as *Pichia* spp., *Candida pulcherrima, Candida lambica, Candida sake, Rhodotorula* spp., and *Debaryomyces polymorphus.* The spoilage moulds associated with fresh-cut fruits include *Penicillium, Aspergillus, Botrytis, Alternaria* and *Rhizopus*. For bacterial spoilage, lactic acid bacteria, *Acetobacter* and *Gluconobacter* are mostly reported (Ray, 2004).

Fresh-cut fruits are also susceptible to various pathogenic microbes such as *Salmonella* spp., *Escherichia coli*, *Shigella sonnei*, *Listeria monocytogenes*, *Cyclospora cayetanensis*, Caliciviruses, Hepatitis A, and Norwalk virus (Harris *et al*., 2003). However, the most reported cases were due to

Salmonella spp. followed by *E. coli* (Harris *et al*., 2003). Salmonella outbreaks are mostly associated with cut melons (Richards and Beuchat, 2005). A typical outbreak of cut melon was happened in Mexico and Central America in 1991 where around 25,000 cases were reported with two fatal incidents (Harris *et al*., 2003). Readers might refer to Harris *et al*. (2003) for a more detail information about food borne illness issues related with fresh-cut fruits.

SYNTHETIC VERSUS NATURAL ANTIMICROBIALS USED IN FRESH-CUT FRUITS

The classification of antimicrobials as synthetic (traditional) versus 'natural' is not easy. This classification does not imply that synthetic or traditional preservatives are less effective than those of the natural origin. Antimicrobials are termed traditional /synthetic when: 1) they have been used for many years, 2) they have been approved by many countries for inclusion as antimicrobials in foods, and 3) they have been produced by chemical synthesis (Raybaudi-Massilia *et al.,* 2009). Ironically, many synthetic traditional antimicrobials such as benzoic acid (in cranberries), citric acid (in lemons), malic acid (in apples) and tartaric acid (in grapes) are found in nature (Raybaudi-Massilia *et al.,* 2009). As mentioned previously, fresh-cut fruits are more susceptible to spoilage and pathogenic microbes than intact fruits (Heard, 2002). Consequently, fresh-cut fruits need more preservative mechanisms than intact and whole fruits. In addition to physical treatments such as modified atmosphere and cold storage, different synthetic preservatives have been used (Table 1). Most of them have inhibitory effect and applied through direct immersion.

Natural plant-derived antimicrobial agents applied to fresh-cut fruits are originated mostly from spices and herbs. The application can be in the powder or liquid form. The liquid extracts mainly containing specific isoprenoids (terpenoids) that belong to essential oils. Essential oils also called volatile oils, are aromatic oily liquids obtained from plant materials such as flowers, buds, seeds, leaves, bark, herbs, fruits, and roots (Burt, 2004). They can be obtained by fermentation, extraction, or distillation with distillation being the most commonly used method for the commercial production of essential oils (Burt, 2004). Essential oils can also be a complex mix of compounds such as terpens, alcohols, cetons, phenols, acids, aldehydes and esters, hydrophobic liquid containing a mixture of antimicrobial volatile aromatic compounds commonly extracted from plant tissues (Burt, 2004; Ayala-Zavala *et al*., 2008; Hamedo, 2009). Application in liquid form is the most common commercial method due to its effectiveness. Different studies suggested the effectiveness of various essential oils from cinnamon, vanilla, clove, citrus peel, lemon, and others against different spoilage and pathogenic microbes (Table 2) (Beuchat, 1996; Lanciotti *et al*., 2004; Raybaudi-Massilia *et al*., 2008a; Raybaudi-Massilia *et al*., 2008b).

The major volatile compounds responsible for the antimicrobial and flavouring property of essential oils include isoprenoids, organic sulfur, aldehydes, and alcohols (Berger, 2007). Isoprenoids are found to be the major scent compounds in cinnamon, coriander, oregano, rosemary, cloves, thyme, and citrus oils. The well-known isoprenoids include citral (3, 7-dimethylocta-2, 6-dienal), and menthol ((1R, 2S, 5R)-5-methyl-2-propan-2-ylcyclohexan-1-ol) (Berger, 2007). Essential oils such as cinammic aldehyde (3-phenylprop-2-enal), citral, eugenol (2-methoxy-4-prop-2-enylphenol), limonene (1-methyl-4-prop-1-en-2-ylcyclohexene), carvacrol (2-methyl-5-propan-2-ylphenol), carvone (2-methyl-5-prop-1-en-2-ylcyclohex-2-en-1-one), and thymol (5-methyl-2-propan-2-ylphenol) have been legally accepted as generally recognized as safe (GRAS) food additivess (Ayala-Zavala *et al*., 2008; Ayala-Zavala *et al*., 2009).

Table 1. Synthetic antimicrobial agents used in fresh-cut fruits to control pathogenic and spoilage microbes

Antimicrobials	Fresh-cut fruits	Target microbes	Concentration and exposure time	References
Ozone	Apples, grapes, oranges, pears, raspberries and strawberries	Many bacteria, moulds and yeast	1–5 ppm, 1–5 min	Khadre *et al*. (2001)
Chlorine-based chemicals (liquid chlorine and hypochlorite)	Many Fresh cut fruits including apples	*Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes*	50–200 ppm, less than 5 min	Burt (2004)
Ascorbic acid	"Gala" apples	*Salmonella* ser. Typhimurium and *E. coli* 0157:H7	3.4 %, 10 min	DiPersio *et al*. (2003)
Sodium metabisulfite	"Gala" appals	*Salmonella* ser. Typhimurium	4.18%, 10 min	DiPersio *et al*. (2003)
Citric acid	"Gala" apples "Valencia" and "Hamlin" orange	*Salmonella* ser. Typhimurium and *E. coli* 0157:H7 Total aerobic microorganism	0.1% , 10 min	DiPersio *et al*. (2003)
Malic acid+ N-acetyl –L-cysteine+ Glutathione + Calcium lactate	"Fuji" apples "Flor de Invierno" pears	*Listeria monocytogenes* and *Salmonella* Enteritidis *E. coli* 0157:H7, mesophilic and psychrophillic bacteria, mould and yeast	0.1% , 1 min	Raybaudi-Massilia *et al*. (2009)
Sodium chlorite (SC) and calcium propionate (CP) (mix)	"Granny Smith" apples	Yeast and mould	0.5% SC and 1% CP, 4-5 min	Guan and Fan(2010)
Hydrogen peroxide	"Golden Delicious" apples	*E. coli* and *Salmonella* spp	5-10 ml/L, 1 min	Abadias *et al*. (2011)

Table 2. Use of natural plant antimicrobial agents in fresh-cut fruits to control pathogenic and spoilage microbes

Essential oil or active compound	Amount (%)	Application method	Fruits	Target microorganisms	References
Cinnamon oil	0.70	Incorporated in to an alginate- based edible coating	Apples and melon	*E. coli* 0157:H7 and *Salmonella* Enteritidis	Raybaudi-Massilia *et al*. (2008a) ; Raybaudi-Massilia *et al*. (2008b)
Citrus oil	0.02	Directly on the fruit skin	A mixture of apple, pear, grape, peach, and kiwi fruit	*E. coli* 0157:H7, *Saccharomyces cerevisiae,* and native micobiota	Lanciotti *et al*. (2004)
Clove oil	0.70	Incorporated in to an alginate-based edible coating	Apple	*E. coli* 0157:H7	Raybaudi-Massilia *et al*. (2008a)
Lemon grass oil	a) 1-0.15 b) 0.70 c) 0.70	Incorporated in to an alginate- based edible coating	a) Apple b) Apple c) Melon	*Listeria innocua* and *E. coli* 0157:H7	Raybaudi-Massilia *et al*. (2008a) ; Raybaudi-Massilia *et al*. (2008b)
Eugenol from Cinnamon	0.50	Incorporated in to an alginate- based edible coating	Apple or Melon	*E. coli* 0157:H7 and *S.* Enteritidis	Raybaudi-Massilia *et al*. (2008a) ; Raybaudi-Massilia *et al*. (2008b)
Hydrosols*	10	Immersion fruits for 20 min	Apple	*Salmonella* Typhimurium and *Escherichia coli* O157:H7	Tornuk *et al*. (2011)

*Hydrosols of thyme, black cumin, sage, rosemary, and bay leaf. Hydrosols are co- or by-products of steam distillation of plant material. Hydrosols contain complex mixtures of essential oils, and phenolic compounds

MODE OF ACTION OF PLANT DERIVED ANTIMICROBIAL AGENTS

The mode of action of natural plant antimicrobials vary and are still not fully understood (Burt, 2004). However, it is suggested that natural antimicrobials have several targets including cell wall deterioration, cytoplasmic membrane injury and leakage of cell contents, membrane protein damage, cytoplasm coagulation, depletion of proton motive force sites, inactivation of essential enzymes and disturbance of DNA and RNA (Burt, 2004; Ayala-Zavala *et al*., 2009).

In this section, only the action of those compounds found in cinnamon and vanilla are described. The antimicrobial activity of cinnamon is mainly due to the presence of cinnamic aldehyde (3-phenyl-2-propenal) and eugenol (2-methoxy-4-(2-propenyl) (Zaika, 1988; Gill and Holley, 2004; Gupta *et al*., 2008; Muthuswamy *et al*., 2008). Gill and Holly (2004) proposed that cinnamic aldehyde could inhibit the energy metabolism by depleting ATP in cells, without disturbing the cell membrane. On the other hand, Gupta *et al*. (2008) proposed that the high electronegative nature of cinnamic aldehyde could interfere the biological process by reacting with nucleic acid and proteins and impede the electron transfer and hence, inhibit the growth of microbes.

Eugenol is a phenol compound and its action is suggested to be analogous to phenolic compounds, which disturbs the cytoplasmic membrane, proton motive force, electron flow, active transport, and coagulation of cell contents in the cytoplasm (Burt, 2004). Vanillin is the essential oil found in vanilla beans. It has similar structure with eugenol and hence, its mode of action is supposed to be similar to that of eugenol (Zaika, 1988; Gupta *et al*., 2008).

Cinnamon

Cinnamon is one of the favourite spices in the world and used as flavouring, preservative agent as well as for its health benefits. Cinnamon contains essential oils and tannins that inhibit microbial growth (Zaika, 1988; Beales, 2002; Gupta *et al*., 2008). According to Rozin and Rozin (1981), cinnamon oil has sweet and fresh aroma that could be compatible with many fresh-cuts such as apple, pear, peach, watermelon, strawberry, grape, lemon, mandarin, orange, grapefruit, pineapple, mango and papaya. Different studies (Table 3) have shown that cinnamon has an antimicrobial effect when added to fresh-cuts and in *in vitro* studies.

The antimicrobial character of cinnamon is mostly due to two major phytochemicals cinnamic aldehyde from the bark (65 to 75%) (Lopez-Malo *et al*., 2000) and eugenol from the leaf (80%) (Ayala-Zavala *et al*., 2008). Zaika (1988) reviewed the antimicrobial strength of different spices and ranked essential oils from cinnamon and clove as the strongest natural antimicrobials. Muthuswamy *et al*. (2008) suggested that cinnamic aldehyde is the main compound responsible for the antimicrobial effect of cinnamon extract.

Apple is one of the most important fruits in the North America so the first part of this discussion will be devoted on fresh-cut apples. Muthuswamy *et al*. (2008) studied the effect of cinnamon bark extract on fresh-cut apples for 12 days at 6^{o}C. This study compared different extraction methods of the cinnamon bark or powder such as water, alcohol and petroleum ether and found that water and petroleum extract did not yield effective antimicrobials. This indicates the effectiveness of alcohol to extract the most active antimicrobial ingredient (cinnamic aldehyde) from cinnamon bark and powder. However, the authors suggested that in the future the extraction should be improved using consumer friendly techniques such as supercritical carbon dioxide extraction instead of alcohol. The *in vitro* studies indicated that ethanol bark extract (2% w/v) had reduced the growth of *E. coli* O157:H7 and *Listeria innocua* by 94 and 87%, respectively (Muthuswamy *et al*., 2008). They also evaluated the effectiveness of cinnamon bark extract in combination with a commercial anti-browning agent, FreshExtend™. The study compared the effect of FreshExtend™ with cinnamon bark extract (1% w/v in 2% ethanol) and FreshExtend™ without cinnamon but ethanol (2% v/v ethanol) by dipping apple slices for 3 min and storing the treated apples at 6°C for 12 days. The authors found that, incorporating cinnamon bark extract (1% v/v) with FreshExtend™ significantly ($p < 0.05$) reduced the growth of aerobic microbes without affecting the color of the cut apples. The results indicated that cinnamon bark extract did not affect the anti-browning capacity of the FreshExtend™. The Whiteness Index (WI) measured the anti-browning effect in this study and it is observed that the WI remains the same for the slices treated with FreshExtend™ plus cinnamon bark extract until 12 day of storage at 6°C. Hence, the study demonstrated the effectiveness of cinnamic aldehyde in reducing the growth of aerobic microbes and the compatibility of cinnamon with the commercial anti-browning solution, FreshExtend™. In this study, the effect of cinnamon treatment on the sensory quality of the fresh-cut apples was not investigated. Even though, Rozin and Rozin (1981), indicated that cinnamon oil has sweet and fresh aroma that could be compatible with sweet tropical fresh-cut fruits, for the practical application of essential oils on fresh-cut fruits the sensory quality should be considered (Ayala-Zavala *et al*., 2008).

Similarly, Raybaudi-Massilia *et al*. (2008a) studied the effect of cinnamon essential oils and cinnamic aldehyde on the microbial and shelf-life of fresh-cut apples. The study found that incorporating cinnamon essential oils (0.7% v/v) and cinnamic aldehyde (0.5% v/v) with alginate-based edible coating significantly reduced ($p < 0.05$) *E. coli* O157:H7 population by more than 4 log CFU/g and extended the microbiological life of the fresh-cuts by 30 days as compared to dipping fresh-cuts in a solution containing only edible coating. This indicates that the cinnamon essential oils and its active ingredient have enhanced the antimicrobial effect of alginate-based edible coating. Nevertheless, the shelf-life was negatively affected by relatively higher concentrations (0.5 and 0.7% v/v) due to the effect of cinnamon extracts on the physicochemical properties (colour, hardness, and flavour) of the fresh-cut apples. Hardness of cut apples has reduced when edible coating was combined with cinnamon essential oils. This is explained by the significant injury of cell wall by the essential oils and their active ingredients. This damage might cause release of calcium and other elements responsible for cell wall integrity and results reduction in firmness. Rojas-Graü *et al*. (2007) reported similar results where pure-alginate edible coating mixed with lemon grass oil (1.5% v/v) and oregano oil (0.5% v/v) has reduced the firmness of fresh-cut apples. Colour was also negatively affected by the application of highly concentrated cinnamon essential oils irrespective of the addition of edible coating, which contains anti-browning compounds such as N-acetyl-L-cysteine and glutathione (Raybaudi-Massilia *et al*., 2008a). Significant reduction ($p < 0.05$) in lightness (L) of apple pieces has been reported by these authors. This means that the enzymatic browning was faster when cinnamon essential oil or active ingredients are mixed with edible coating. The anti-browning effect of the edible coating might be affected by cinnamon due to the significant damage of the cellular membranes, which releases enzymes such as polyphenol oxidase and substrates for the enzymatic browning reaction. This study has also found that the sensory quality was unfavourably affected by the addition of higher concentration of cinnamon extracts.

Raybaudi-Massilia *et al*. (2008_b) conducted a similar study with melon and reported significant reduction ($p < 0.05$) of *Salmonella enteritidis* and extended microbiological shelf-life by more than 21 days when cinnamon essential oils and their active compounds are incorporated with edible coating. They also indicated the same problem on the physicochemical property of the fresh-cuts when higher concentration of essential oils is added. Roller and Seedhar (2002) examined the effect of cinnamic acid on cut melon and kiwi fruit and found that dipping the fresh-cuts for one min in solutions containing 1 to 15 mM cinnamic acid is effective in extending the shelf-life of the fresh-cut fruits for 21 days at 4°C by inhibiting the viable flora. Even if higher concentrations were more effective, undesirable pungency and colour change has been observed on the fresh-cut fruits and hence the authors recommended 1 mM of cinnamic acid for achieving safety and quality. Since this study was done in sealed jars, the result may not be extrapolated for fresh-cut fruits packed in semi-permeable plastic films, which could be more common in the market place. In addition, this study did not show the effect of cinnamic acid on specific spoilage and pathogenic microbes rather they did total viable count. Hence, further study might be necessary to include different packaging and counting specific microbes for the target fruit.

Although most current researches focused on pathogenic bacteria, Bullerman (1974) demonstrated that cinnamon essential oils at 200 ppm and cinnamic aldehyde at 150 ppm were inhibitory for growth of *Aspergillus parasiticus* and the resulting aflatoxin production *in vitro.* Baratta *et al*. (1998) also studied the effect of cinnamon essential oil against the common spoilage fungi *Aspergillus niger* and found that cinnamon oil at concentration of 1 µL/mL could inhibit the growth of the spoilage fungus.

Table 3: Use of cinnamon-derived antimicrobial agents to control pathogenic and spoilage microbes on different fresh-cut fruits and *in vitro*

Inhibited microbes	Fruit	Minimum inhibitory concentration (MIC*) and compound	References
Viable count of natural flora	Cut melon and kiwi fruit	1-15 mM cinnamic aldehyde	Roller and Seedhar (2002)
Salmonella Enteritidis	Cut melon	Cinnamon essential oil 0.7% and cinnamic aldehyde 0.5%	Raybaudi-Massilia *et al*. (2008b)
Escherichia coli O157:H7	Cut apple	Cinnamon essential oil 0.7% and cinnamic aldehyde 0.5%	Raybaudi-Massilia *et al*. (2008a)
E. coli O157:H7 and *Listeria innocua*	Cut apple	2.95 mM cinnamon essential oil	Muthuswamy *et al*. (2008)
S. Enteritidis, *E. coli,* and *L. innocua*	Apple and pear juices	2 µL/mL for pear juice 8-10 µL/mL for apple juice cinnamon essential oil	Raybaudi-Massilia *et al*. (2006)
E. coli O157:H7 and *S. typhimrium*	*In vitro*	3 mM cinnamon essential oil	Helander *et al*. (1998)
Bacillus subtilis, Enterobacter aeruginosa, E. coli, Staphylococcus aureus, and *Staphylococcus epidermis*	*In vitro*	250 µg/mL cinnamon essential oil	Al-Saghir (2009)
Aspergillus parasiticus	*In vitro*	150-200 ppm cinnamic aldehyde	Bullerman (1974)
Listeria monocytogenes	*In vitro*	30 mM cinnamic aldehyde	Gill and Holly (2004 and 2006)
Bacillus spp, *L. monocytogenes, E. coli,* and *Klebsiella* spp, *Rhizomucor* spp	*In vitro*	1.25% v/v cinnamon essential oil	Gupta *et al*. (2008)
Colletortrichum coccodes, Botrytis cinerea, Cladosporium herbarum, Rhizopus stolonifer, and *Aspergillus niger*	*In vitro*	25-500 ppm cinnamon essential oil	Tzortzakis (2009)
A. niger	*In vitro*	1µL/ mL cinnamon essential oil	Baratta *et al*. (1998)

*Minimum inhibitory concentration (MIC) is defined as the lowest concentration that completely inhibit the growth for 24 hr (Thongson *et al*., 2004)

Recently, Tzortzakis (2009) studied the effect of cinnamon oil on fungus such as *Colletotrichum coccodes, Botrytis cinerea, Cladosporium herbarum, Rhizopus stolonifer* and *Aspergillus niger in vitro* and found that 25 ppm essential oils inhibited 63% of fungal spore and at 500 ppm, fungal sporulation was completely hindered.

Hence, recent and earlier studies suggested that cinnamon has wide antimicrobial activity against gram-positive and gram-negative bacteria as well as yeasts and moulds.

Vanilla

The incorporation of vanilla is common in ice cream, beverages, biscuits, chocolate, and other disserts. Vanillin (4-hydroxy-3-methoxybenzaldehyde) is low molecular weight phenolic antimicrobial compound extracted from vanilla (Fitzgerald *et al*., 2003). Vanillin has been widely used by the food industry as GRAS product and its organoleptic property is well accepted by many consumers (Rao and Ravishankar, 2000). Vanillin has broad antimicrobial action against gram-positive and gram-negative bacteria, moulds and yeasts (Table 4). Different researches investigated its effect on the microbial quality of fresh-cut fruits as discussed below.

Rupasinghe *et al*. (2006) studied the effect of vanillin combined with an anti-browning compound called NatureSeal™ on apple slices in two apple cultivars at 4°C for 19 days. NatureSeal™ is a patented product composed of vitamins and minerals and used to extend the shelf-life of fresh-cut fruits by inhibiting enzymatic browning and softening, but do not have antimicrobial activity (NatureSeal, 2009). In this study, 12 mM vanillin combined with NatureSeal™ could reduce the total microbial load by 37% for "Crispin" apples and 66% for "Empire" apples. The study also tested the effect of vanillin on eight selected microbes *in vitro* and found that vanillin at concentration of 8 to 18 mM could inhibit the growth of *E. coli, Pseudomonas aeruginosa, Enterobacter aerogenes, Salmonella enterica* subsp. *enterica* serovar Typhimurium, *Candida albicans, Lactobacillus casei, Penicillium expansum,* and *Saccharomyces cerevisiae.* The study also showed that vanillin combined with NatureSeal™ could significantly reduce ($p < 0.05$) the microbial load without affecting the colour and firmness of the fresh-cut apples. This could indicate that incorporation of vanillin did not affect the properties of NatureSeal™ to act as an anti-browning and anti-softening agent. However, this study did not show the effect of vanillin on the sensory quality of apple pieces, which may be necessary before applying the finding in to the commercial products. In this study, it was observed that *Penicillium expansum* inhibition needs higher concentration (18 mM) of vanillin, which might affect the sensory quality of the cut fruits. On the other hand, the same study demonstrated that lower concentrations (1 to 3 mM) could enhance the growth of *Saccharomyces cerevisiae*, which might lead greater fungal infection of cut-apples. The enhanced growth of *S. cerevisiae* was explained by Fitzgerald *et al*. (2003) due to bioconversion of vanillin in to vanillyl alcohol and vanillic acid by *S. cerevisiae*, which is advantageous for the organism.

Another study has been done using a different apple variety and alginate-apple puree edible coating. Rojas-Graü *et al*. (2007) studied the effect of vanillin by combining with a coating compound namely alginate-apple puree edible coating at concentration of 0.3 to 0.6% on apple pieces from a variety *Malus domestica* Borkh. cv. Fuji. The coating is prepared from apple puree-alginate solution with the addition of vanillin. Calcium chloride and N-acetylcysteine was added to all vanillin treated cuttings. The study found significant ($p < 0.05$) reduction of psychrophilic aerobic bacteria, yeasts and moulds after adding vanillin to the coating mix at the given concentration. Similar to the findings of Rupasinghe *et al*. (2006), addition of vanillin did not affect the colour of apple pieces and hence, the ability of N-acetylcysteine to act as anti-browning agent was not affected by vanillin added at 0.3 to

0.6%. This study also showed that apple pieces treated with vanillin plus apple-puree alginate coatings maintained their firmness during the 21 days storage time. This finding is in agreement with that of Rupasinghe *et al*. (2006) who reported that vanillin addition to NatureSeal™ maintained firmness. In this study, a sensory study was conducted and found that incorporation of vanillin resulted in acceptable scores during the examined storage time.

Another study by Hun-Sik Chung *et al*. (2009) has shown significant effect of vanillin on inhibition of total antimicrobal count, yeasts and moulds on apple cuttings when it is applied at concentraions ranging from 40 to 120 mM on *Malus domestica* Borkh. cv. Fuji. In this study, treated cuttings were stored at 4^{o}C for 3 weeks and antimicrobial effect of vanillin was strong throughout the storge period. As opposed to Rupasinghe *et al*. (2006) and Rojas-Graü *et al*. (2007), this study indicated that addition of vanillin yielded undesirable browning and unacceptable test quality. This undesirable effect could be due to high concentration (80 to 120 mM) of vanillin used in this research. However, this might suggest that application of the correct concentration of vanillin is important for maintaining the sensory quality. In addition to apple, the effect of vanillin on tropical fruits has been investigated (Ngarmsak *et al*., 2006; Sangsuwan *et al*., 2008). According to Ngarmsak *et al*. (2006), dipping mango pieces in vanillin (80 mM) at 5 to 10^{o}C, significantly reduced ($p < 0.05$) total bacterial count as well as yeast and mould population for 14 days. Sangsuwan *et al*. (2008) investigated the effect of vanillin on cut melon and pineapple by preparing a vanillin film (chitosan/methyl containing vanillin) and found that cut pineapple and cut melon wrapped with vanillin film had the lowest yeast and bacterial population as compared to other films (commercial stretch film and chitosan/methyl without vanillin). The effect of vanillin against *E. coli* and *Saccharomyces cerevisiae* was different for pineapple and melon. Sangsuwan *et al*. (2008) explained that vanillin was more efficient in pineapple than melon due to the lower pH of pineapple. López-Malo *et al*. (1998) also reported that growth of *Aspergillus flavus* and *Aspergillus niger* was inhibited after adding vanilla at lower pH (3 to 4) than higher pH values at a given concentration. Presser *et al*. (1997) further confirmed that growth of *E. coli in vitro* was inhibited at lower pH (3.7) than higher pH (4.0). This indicates the combined positive effect of low pH and vanillin. Unlike to the previously discussed studies, these authors studied the effect of vanillin on the nutritional quality of fresh-cut pineapple and melon. The study found that ascorbic acid content in pineapple wrapped with vanillin film has been reduced significantly ($p < 0.05$) and only 10% of the original concentrations left after 12 days of storage. Therefore, the reaction of vanillin with ascorbic acid compounds should be examined in the future.

In all the reviewed studies, different concentrations of vanillin and cinnamon constituents were used. The difference in concentration depends on various factors such as type of fruit, the target organism and the pH of the fruit. A fruit with high protein or fat content explain the necessity of a higher concentration to obtain the same effect (Lopez-Malo *et al*., 2000). Ayala-Zavala (2008) indicated that the activity of natural antimicrobials could be decreased due to its reaction with proteins, lipids and carbohydrates.

Table 4. Use of Vanillin as antimicrobial agents to control pathogenic and spoilage microbes on different fresh-cut fruits and *in vitro*

Inhibited microbes	MIC*	Fruit	Reference
Escherichia coli, Pseudomonas aeruginosa, Enterobacter aerogenes, Salmonella enterica subsp. *enterica* serovar, *Candida albicans, Lactobacillus casei, Penicillum expansum,* and *Saccharomyces cerevisiae*	6-18 mM	"Empire", "Crispin" apple	Rupasinghe *et al*. (2006)
Psychrophilic aerobes, yeasts and moulds	0.1% w/w	"Fuji" apples	Rojas-Graü *et al*. (2007)
Total aerobic bacteria, yeast and mould	0, 40, 80, 120 mM	"Fuji" apples	Hun-Sik Chung *et al*. (2009)
Total bacterial count, yeast and mould	40-80 mM	Mango, pineapple	Ngarmsak *et al*. (2006)
E. coli and total yeast count	40-50 µm (film thickness)	Cantaloupe, pineapple	Sangsuwan *et al*. (2008)
S. cerevisiae, Penicillium membranaefaciens, Zygosaccharomyces bailii, and *Saccharomyces bailii*	40-80 mM	Strawberry puree	Cerrutti and Alzmora (1996)
S. cerevisiae, and *Zygosaccharomyces rouxii*	21, 20, 13 mM	*In vitro*	Fitzgerald *et al*. (2003)
S. cerevisiae, Z. bailii, Z. rouxii, and *Debaryomyces hansenii*	2000 ppm	*In vitro,* apple puree	Cerrutti *et al*. (1997)
Total aerobic and anaerobic bacteria, yeast and mould	3000 ppm	Banana puree	Castanon *et al*. (1999)
Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, and *Aspergillus parasiticus*	500-1000 ppm	*In vitro*	Lopez-Malo *et al*. (1998)
Penicillium digitatum, Penicillium glabrum, and *Penicillium italicum*	1000-1300 ppm	*In vitro*	Matamoros-Lessential oilsn *et al*. (1999)
S. cerevisiae, and *Candida parapsilosis*	13, 20, 21 mM	Fruit juice, soft drink	Fitzgerald *et al*. (2003)

*Minimum inhibitory concentration (MIC) is defined as the lowest concentration that completely inhibit the growth for 24 hr (Thongson *et al*., 2004)

CONCLUSIONS AND FUTURE PROSPECTS

This article reviewed the effect of selected natural plant-derived antimicrobial agents on many pathogenic and spoilage microorganisms with special emphasis on the use of natural compounds of vanilla and cinnamon. Several studies have confirmed that the antimicrobial potential of cinnamon and vanilla extracts in controlling or preventing the growth of pathogenic and spoilage microbes in fresh-cut fruits. Cinnamic aldehyde and vanillin are identified to be the major antimicrobial agents of cinnamon and vanilla, respectively. It has also been shown that vanilla and cinnamon compounds are effective when applied alone or incorporated with edible coatings, which are mostly added for their anti-browning and hardening effect. However, some studies have indicated that unless appropriate concentrations are used to treat fresh-cut fruits, undesirable flavour and texture can be resulted. Hence, determining the optimum concentration and undertaking sensory tests is advisable while investigating for new natural antimicrobial agents. It has indicated the significant reduction of ascorbic acid content in cut pineapple treated with vanillin. Hence, further research is warranted on the impact of vanillin on metabolism of fresh-cuts. In all the reported research models, whole fruits were washed by an antimicrobial solution before cutting into pieces; hence, it might be difficult to conclude that the inhibition effect is only from the natural antimicrobial agents. Further research should also be conducted under the industrial setting of fresh-cut processing.

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