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Research Article

Antimicrobial effects of vanillin coated solution for coating paperboard intended for packaging bakery products

Suwarat Rakchoy, Panuwat Suppakul and Tunyarut Jinkarn*

Department of Packaging and Materials Technology, Faculty of Agro-industry, Kasetsart University, Bangkok, Thailand

* Author to whom correspondence should be addressed, email: fagitvp@ku.ac.th

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Abstract

Paperboard is commonly used for distribution and sales packaging of bakery products. Typical bakery products have unique flavour characteristics that could be impaired by volatile antimicrobial compounds contained in the packaging material resulting in unacceptable product quality. This study investigated the antimicrobial effects of vanillin solutions for coating paperboard intended for packaging bakery products. Three coating solutions were evaluated: vanillin/dimethyl sulfoxide (DMSO) (10, 5, 2.5 and 1.25% (w/w)), vanillin/ethyl alcohol (10, 5, 2.5 and 1.25% (w/w)) and vanillin/chitosan (10, 5, 2.5, 1.25, 0.625 and 0.3125 % (w/w)). The inhibitory effects of all coating solutions were investigated against three types of common pathogens and food spoilage bacteria: *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus* using the agar well diffusion method. Significant inhibitory effects for all bacteria were observed for all coating solutions. For vanillin/DMSO and vanillin/alcohol, the inhibition was more effective over *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* respectively with a minimum inhibitory concentration (MIC) at 2.5%. Furthermore, the MIC for vanillin/chitosan coating solution was 5% (w/w) for both *Escherichia coli* and *Staphylococcus aureus*. However, the MIC of vanillin/chitosan for *Bacillus cereus* was only 0.625% (w/w). For vanillin/chitosan, MICs for certain bacteria might depend on the inhibitory effectiveness and amount of vanillin and chitosan in the coating solution.

Keywords: vanillin, antimicrobial, paperboard packaging, bakery products, Thailand

Introduction

Bakery products are considered perishable products and are composed of heterogeneous mixtures such as eggs, flour, butter, milk and sugar. These products are the best nutrient sources for microbial growth and thus have a very short term shelf-life. As a result, bakery products packaging materials must provide both barrier and protective functions, more so than the packaging for other durable products. At present, many approaches have been proposed for controlling microbial growth in food. Active packaging technologies are designed to extend the shelf-life of food, while maintaining nutritional quality and safety. Antimicrobial agents may be incorporated into the packaging materials and migrate into the food through diffusion and partitioning [1].

Paper and paperboard are the most widely used materials in food and drink packaging [2, 3]. Furthermore, paper as well as plastics can be used as antimicrobial packaging materials. Because paper has a porous structure, the antimicrobial agents plugged in the pores may improve the paper material's performance, such as water vapour and gas permeability, physical strength, optical properties, and surface properties, as well as the antimicrobial activity [1]. Constituents of antimicrobial agents of packaging materials can diffuse into food products and these migration processes can extend the shelf-life of the packaged product. In addition, these agents improve the safety or sensory properties of food while maintaining its quality [4, 5].

Modern consumer opinion suggests a desire for high quality food that is more natural, minimally processed and preservative free, while remaining safe and with an extended shelf life [6]. This has driven the food industry and food research towards the search for natural antimicrobial compounds such as native food preservative ingredients, herbal extractions, or antimicrobial agents derived from animals.

Since vanillin is a familiar flavour for bakery products, this study aims to develop antimicrobial paperboard containing vanillin for shelf life extention, as well as for providing favourable aroma paper packages from vanillin. Vanillin or *4-hydroxy-3-methoxybenzaldehyde*, is an organic compound with the molecular formula $C_8H_8O_3$. Its functional groups include aldehyde, ether and phenol. It is the primary component of the extract of the vanilla bean. Vanillin is considered to be one of the most widely appreciated flavour compounds, with an odour threshold for humans equal to 11.8×10^{-14} M, and has the unique characteristic that, even at high doses, the flavour is still pleasant [7] and it is generally regarded as a safe (GRAS) flavouring compound. It is used widely in ice cream, beverages, chocolate, cakes, many baked products, confectionary and desserts, etc. [8, 9, 10] and also used in the fragrance industry and pharmaceutical formulations. Furthermore, vanillin exhibits several bioactive properties [11] e.g. antioxidant [12, 13] and antimicrobial activities against yeasts, moulds [14, 3] and bacteria [15, 16]. In the antimicrobial packaging area, vanillin has been used to develop antimicrobial film [9], however, no publication indicates the use of vanillin in the paper packaging or materials.

Vanillin has also been reported to possess anticlastogenic, antimutagenic and antitumor properties and, therefore, it can be considered as a nutraceutical molecule [10, 17]. The antimicrobial property of vanillin is the effect of phenolic compound in chemical structure which makes vanillin effective in inhibiting bacteria, yeasts and moulds. It is structurally similar to eugenol (2-methoxy-4-(2-propenyl) phenol) from cloves and is known to be antimycotic [8] and bacteriostatic [18]. At low concentrations, phenols affect enzyme activity, especially those enzymes associated with energy production, while at greater concentrations they cause proteins to denature [19]. Additionally, some research has indicated that vanillin was more effective for low pH products at the higher temperature condition [9, 13].

For this study, different vanillin solutions were prepared. These mixtures were intended for further use in coating paperboard intended for packaging bakery products. The objectives of this part of the study were to evaluate the inhibitory effects of vanillin coated solutions and to investigate minimum inhibitory concentration (MICs) of the solutions against selected bacteria including *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*.

Materials and Methods

Reagents and solvents

Vanillin was obtained from Sigma-Aldrich (St. Louis. MO, USA), Dimethylsuloxide (DMSO) and Chitosan 95% DAC, MW~760000 were obtained from Sigma-Aldrich (Laborchemikalien GmbH, Germany) and Seafresh Chitosan Lab Company Limited (Thailand), respectively. Ethanol 95%, lactic acid and glycerol were obtained from Merck (Germany), Ajax Finechem (Australia) and J.T. Baker (USA), respectively.

Bacterial stains and culture preparations

Three types of common pathogenic and food spoilage bacteria were selected for their relevance in bakery products and other food: the gram-positive bacteria; *Staphylococcus aureus* (DMST 8840) and *Bacillus cereus* (DMST 5040) and the gram negative bacteria; *Escherichia coli* (DMST 4212). All bacterial strains were provided by the Department of Medical Sciences, Thailand.

Preparation of vanillin solutions

Three types of coated solution were prepared including vanillin/DMSO; vanillin/ethyl alcohol and vanillin/chitosan. Vanillin/DMSO and vanillin/ethyl alcohol solutions were prepare by diluting vanillin (>99% purity) directly with DMSO and with 95% ethyl alcohol respectively, in a series of half-fold dilutions, 10%, 5%, 2.5% and 1.25% (w/w). For vanillin/chitosan coated solution, preparation was composed of two steps. First, 1% chitosan solution was prepared by dissolving one gram of chitosan in 1% lactic acid solution. During this process, one gram of glycerol was added as a plasticizer. Finally, pure vanillin was diluted with 1% chitosan solution which was prepared previously in a series of half-fold dilutions, 10%, 5%, 2.5%, 1.25%, 0.635% and 0.3125% (w/w).

Antimicrobial testing

The inhibitory activities of vanillin solutions: vanillin/DMSO, vanillin/ethyl alcohol and vanillin/chitosan were analyzed by a modified agar-well diffusion technique as described by Chung *et al*. [20]. Plates were filled with 100 µl of standard 10^6 Colony Forming Unit (CFU)/ml of microorganisms and then 15 ml of Nutrient agar (Merck, Germany) was added and mixed together by a pour plate technique. When the medium was solidified, using a sterile cork borer, wells of 5.0 mm. in diameter were made and filled with 40 µl of vanillin mixtures. DMSO, 95% ethyl alcohol and 1% chitosan in lactic acid were used as control solutions. The plates were incubated for 18 to 24 h at 37^oC. In order to obtain comparable results, all prepared solutions were treated under the same conditions under the same incubated plates. All tests were performed for three replicates. The inhibitory activities of the vanillin testing solutions were detected as clear zones around the wells and diameters of clear zones were expressed in millimeters (mm). Minimum inhibitory concentrations (MICs) which were defined as the lowest concentrations of particular vanillin coated solutions that can inhibit certain bacteria were also investigated.

Results and Discussion

The average diameter of inhibition clear zone of all vanillin solutions are illustrated in Figure 1. Significant inhibitory effects for all bacteria were observed for all vanillin solutions. Further, clear zone diameters increased with regard to the increasing vanillin concentration for all types of vanillin solutions (Figures 2-4).

The antimicrobial activity of vanillin depends on the time of exposure, concentration, the target organism and conditions of the solution or processing (e.g. pH and temperature) [9]. Recent reports show that vanillin has been used to inhibit *E. coli* in 'Granny Smith' apple juice [21]. Rupasinghe *et al.*[16] also reported that 6-18 mg of vanillin can effectively inhibit *E. coli* (ATCC 25922) in fresh-cut 'Empire' and 'Crispin' apple slices. Also, 30 mg of vanillin per 100 ml of inoculated carrot broth completely inhibited *Bacillus cereus* (INRA L2104) spores for more than 60 days at 16°C [22].

For this study, vanillin/DMSO and vanillin/alcohol was more effective over *E. coli, S. aureus* and *B. cereus* respectively, with a minimum inhibitory concentration (MIC) at 2.5% (w/w). The results agree with previous studies on the effect of vanillin in inhibiting *E. coli* and *B. cereus.* In addition, results are also in accordance with Mourtzinos *et al*. [13] who reported that *E. coli* is more sensitive to vanillin than *B. cereus* and *S. aureus*.

According to the results, the MIC for vanillin/chitosan coated solution was at 5% (w/w) for both *E. coli* and *S. aureus*. However, the MIC of vanillin/chitosan for *B. cereus* was only 0.625% (w/w).The antimicrobial activity of the vanillin/chitosan might depend on the inhibitory effectiveness and amount of vanillin and chitosan, as well as influence of lactic acid in the coating solution.

Chitosan is inherently antimicrobial. Although studies on the effect of chitosan and vanillin on *B. cereus* were limited, the inhibiting effect of vanillin or chitosan on other bacteria such as *E. coli*, *Listeria monocytogenes* and *Saccharomyces cerevisiae* were found in a number of studies. Brody *et al*. [23] reported that the antimicrobial effect of chitosan occurs when organisms are in direct contact with the active sites of chitosan. Zivanovic *et al*. [24] applied the mixture of chitosan, oregano and essential oil and compared this with pure chitosan films on inoculated bologna meat samples stored for 5 days at 10^0C. The result showed that both samples were effective in inhibiting *L. monocytogenes* and *E. coli*. According to the study by Sangsuwan *et al*. [9] on the antimicrobial effectiveness of chitosan/methyl cellulose film compared with vanillin film against *E. coli* and *S. cerevisiae* on fresh-cut fruit, results revealed that both films exhibited inhibitory effect against *E. coli* on fresh-cut cantaloupe. The vanillin film was more efficient than chitosan/methyl cellulose in reducing the volume of yeast, which decreased by 4 logs in fresh-cut pineapple on day six.

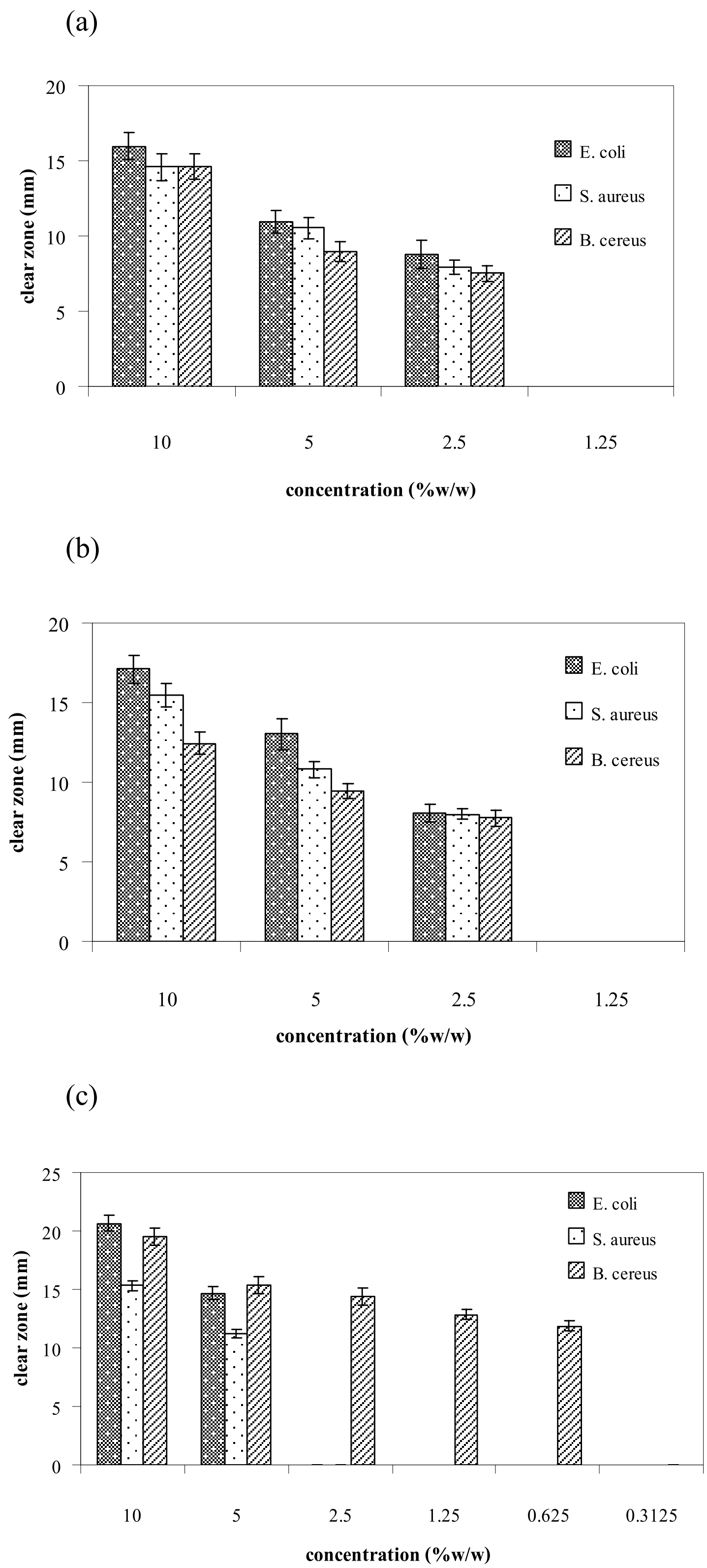
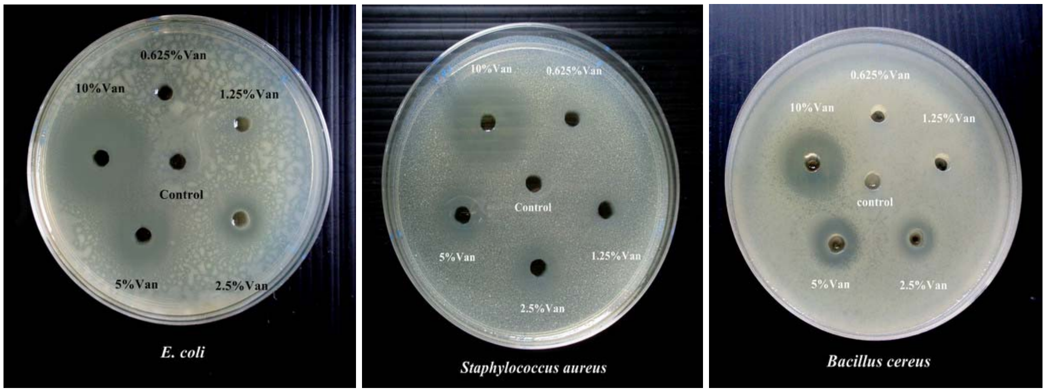
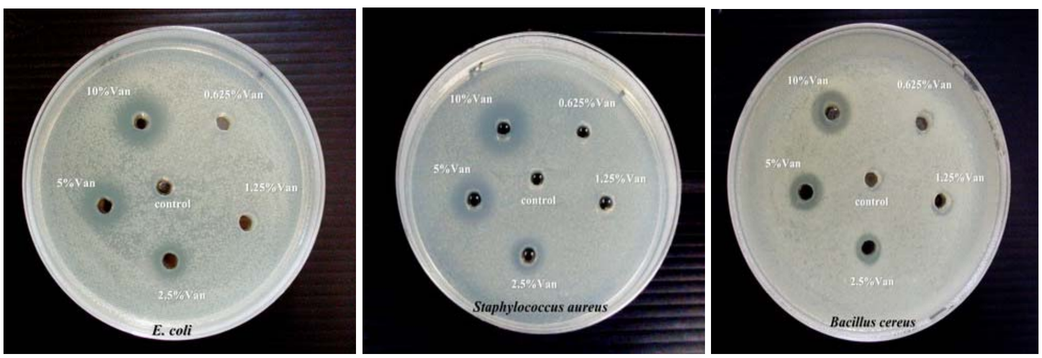


Figure 1. Antimicrobial activities and MICs of vanillin coated solutions.
(a) Vanillin/DMSO (b) Vanillin/ethyl alcohol (c) Vanillin/chitosan



(a) (b) (c)

Figure 2. Clear zones of vanillin/DMSO in inhibiting tree types of bacteria.
(a) *Escherichia coli*, (b) *Staphylococcus aureus* (c) *Bacillus cereus*



(a) (b) (c)

Figure 3. Clear zone of vanillin/ethyl alcohol in inhibiting tree types of bacteria.
(a) *Escherichia coli*, (b) *Staphylococcus aureus* (c) *Bacillus cereus*

For this study, 1% of chitosan solution was used as the control and the results showed no inhibitory effects. This result agrees with Pranoto *et al*. [25] who incorporated antimicrobial agents in chitosan films. Activities of the antimicrobial films were tested for food pathogenic bacteria including *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*. Results revealed that pure chitosan film had no inhibitory effect. However, in this study, *B. cereus* (MIC 0.625 % (w/w)) was more sensitive to vanillin/chitosan than *E. coli* and *S. aureus* (MICs 0.5 % (w/w)). This could be explained by *B. cereus* producing the highest amount of chitosanase, the active enzymes that can hydrolyze chitosan [26]. Purified chitosanase has been previously used in the preparation of chitosanoligosaccharides from chitosan. These oligosaccharides showed antimicrobial activities [27, 28, 29]. As a result, antimicrobial inhibitory of vanillin/chitosan solution against *B. cereus* is more effective compared with other microorganisms under the study.

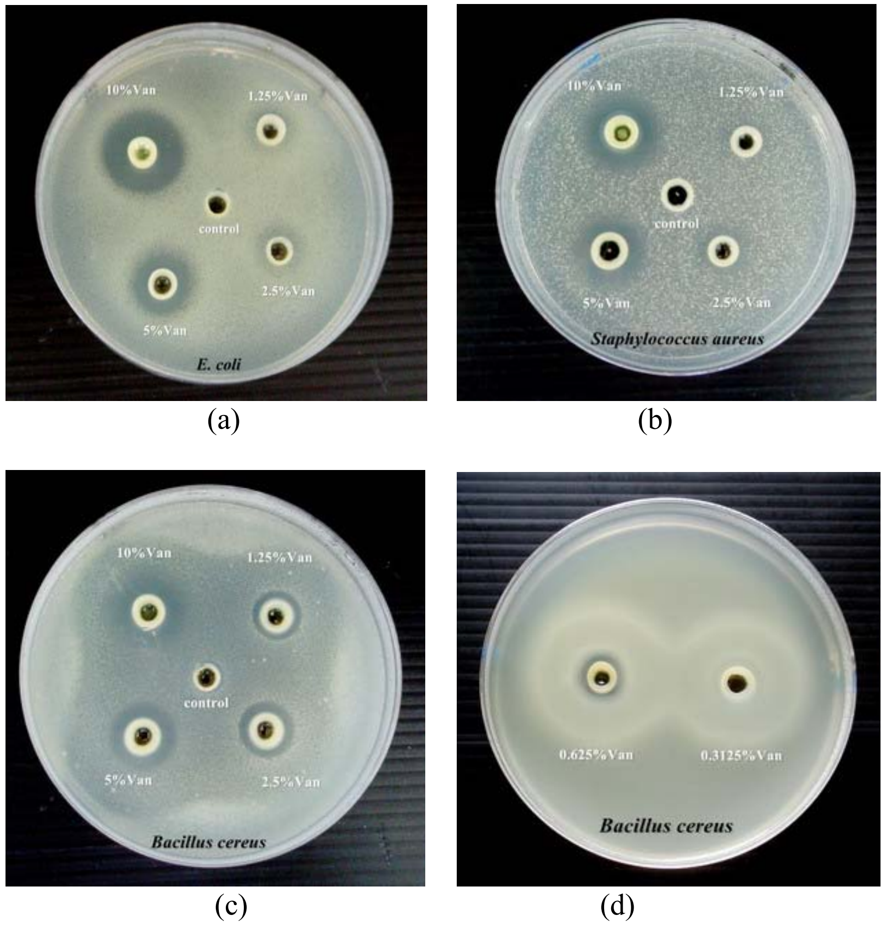


Figure 4. Clear zone of vanillin/chitosan in inhibiting three types of bacteria.
(a) *Escherichia coli*, (b) *Staphylococcus aureus* (c, d) *Bacillus cereus*

For vanillin/chitosan coated solution that has the MICs at 5 % for both *E. coli* and *S. aureus*, this could also be due to the effect of chitosan in the mixture that might prohibit the inhibitory effectiveness of vanillin, resulting in a higher MIC than other vanillin mixtures.

Conclusions

Vanillin is a promising antimicrobial agent for coating paper packaging of bakery products. This study revealed that vanillin coated solutions significantly inhibit three types of common food pathogenic and spoilage bacteria: *E. coli*, *S. aureus* and *B. cereus*. However, the inhibitory effect of coated solutions for specific types of bacteria depends on the concentrations of vanillin, the combination of substances in the mixture and the mixtures concentration.

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