Research Article

Antimicrobial effects of coating solution containing clove oil and hydrophobic starch for coating paperboard
Sudsuda Vanit, Panuwat Suppakul and Tunyarut Jinkarn*

Department of Packaging and Materials Technology, Faculty of Agro-Industry, Kasetsart University, Bang Khen, Bangkok, Thailand.

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

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Abstract

Since paperboard is one of the widely used packaging materials, its properties have been extensively studied, especially its physical and mechanical properties to provide better product protection and packaging performance, whereas studies related to antimicrobial properties of the various boards are limited. This study focused on developing antimicrobial coating solutions for paperboard containing a natural plant extract clove oil (Syzygium aromaticum) in a modified hydrophobic starch matrix. The inhibitory effects of three coating solutions composed of pure clove oil and clove oil in 5% and 8% (w/w) modified starch matrix were investigated. Hydrophobic starch was selected for this study. For pure clove oil, clove oil was diluted with dimethylsulphoxide (DMSO) in a series of half-fold dilutions, 5%, 2.5%, 1.25%, 0.625% and 0.1325% (v/v). For coating solutions in modified hydrophobic starch matrix, the suspensions of starch at 5% and 8% (w/w: dry weight basis) were heated in order to form a gel and clove oil was added at 5%, 2.5%, 1.25%, 0.625% and 0.3125% (w/w) respectively, then homogenized. The inhibitory effects of all coating solutions against three types of common pathogens and food spoilage bacteria were investigated including Escherichia coli, Bacillus cereus and Staphylococcus aureus, using the agar well diffusion method. Pure clove oil showed a minimum inhibitory concentration (MIC) of 1.25% against the growth of Escherichia coli while the MIC for the other two bacteria (Bacillus cereus and Staphylococcus aureus) was at 2.5%. In addition, a coating solution containing clove oil in 8% hydrophobic starch matrix showed better inhibiting effect than in 5% hydrophobic starch matrix over all types of bacteria with the MICs similar to pure clove oil solution at 1.25% for Escherichia coli and at 2.5% for Bacillus cereus and Staphylococcus aureus.
Keywords: Syzygium aromaticum, antimicrobial, clove oil, paperboard, coating, packaging, Escherichia coli, Bacillus cereus, Staphylococcus aureus, Thailand

Introduction

Paper and paperboard have been increasingly used as a packaging material since they are renewable, recyclable and biodegradable. More than 2.5 million tons of paper and paperboard are used worldwide in food packaging [1]. However, food packed in paper and paperboard can be contaminated with microorganisms such as Bacillus cereus or Escherichia coli [2]. Packaging for food not only provides a long shelf life and good protection, but also prevents food from various risks including microbial spoilage. At present, antimicrobial packaging materials are being developed to solve many problems associated with food distribution and safety [3, 4, 5, 6, 7, 8]. In fact, antimicrobial packaging is considered as active packaging that can be defined as a mode of packaging in which the package, the product and the environment interact to prolong shelf life or enhance safety or sensory properties, while maintaining the quality of the product [9].

The demand for safer and more natural food has been increasing since consumers have become more concerned with chemical residues in food, so natural compounds such as spices and herbal oils are alternatives to be used as food preservatives since they have antimicrobial or antifungal properties. Furthermore, they do not have any significant medical or environmental impact [3]. Clove oil (Syzygium aromaticum) is an essential oil which has antimicrobial properties against important human pathogenic microorganisms and microorganisms that cause food spoilage due to its active ingredient, eugenol [10, 11].

Clove oil has been selected as an antimicrobial agent for various packaging materials and systems. Rodriguez, et.al. [3] assessed the antimicrobial and vapour-phase activity of natural essential oils (EOs) including clove (Syzygium aromaticum), cinnamon (Cinnamomum zeylanicum), oregano (Origanum vulgare) and cinnamaldehyde-enriched cinnamon when used in paraffin-based “active coatings” for paper packaging materials intended to come into contact with food. Coma [12] studied the antimicrobial packaging system on meat products and several studies have also been performed on antimicrobial packaging materials containing clove oil [13, 14, 15].

Starch, a natural polysaccharide, has been extensively used in the paper industry to increase the sheet dry strength and retain fragments of fibres. Starch is also applied to dry sheet to increase the water penetration resistance into paper [16]. However, natural starch also has some drawbacks such as its high viscosity which is not suitable for coating. In addition, the greater stability of the molecules of the modified starch and their inherently better theology make them useful at higher molecular weights without runnability problems. These starches also have a greater binding power compared to unmodified starches [17]. Therefore, modified starch which shows better performance for coating tends to be used in paper industry. Particular modified starches are utilized in paper production such as cationic starch and oxidized starch [18]. However, utilization of another modified starch, hydrophobic starch, is increasing for the paper industry because hydrophobic starches can provide water, oil and grease resistance with superior film-forming properties [19].

Since modified starches are common coating substances in most paper mills, it is possible to combine such coating substances with antimicrobial agents to develop an antimicrobial paper
with no need for extra coating or laminating process. Starch is not only used as an antimicrobial carrier, it also improves the mechanical and surface properties of the coated paper. There are also a few studies that focus on coating paper packaging materials with biopolymer substances containing antimicrobial agents such as by Arfa and colleagues [20, 21] that prepared antimicrobial papers by coating the paper with soy protein isolate (SPI) solution as an inclusion in a matrix of carvacrol, and the study by Tepsorn [22], that investigated the antimicrobial activity of various Thai medicinal plants in alginate-tapioca starch based edible films.

This study aimed to investigate the antimicrobial effect of paper coating solutions composed of clove oil in hydrophobic starch matrixes at different levels of clove oil and starch concentration. Results from this study can help to prepare effective paper coating solutions for antimicrobial paper development.

Materials and Methods

Microbial strains
Microorganisms were obtained from the Department of Medical Sciences, Thailand. The organisms used comprised of two Gram-positive organisms (Bacillus cereus DMST 5040 and Staphylococcus aureus DMST 8840) and a Gram-negative bacterium (Escherichia coli DMST 4212).

Essential oils
Clove bud oil (98% eugenol) was provided by Sigma-Aldrich, Singapore. The oil was diluted in a half-fold dilution series with dimethylsulphoxide; DMSO (Sigma-Aldrich, Germany) to achieve a decreasing concentration range of 5.0000% to 0.3125% (v/v).

Hydrophobic starch
Modified starch selected for this study was the hydrophobic starch (FILMKOTE®250). The starch was supplied by National Starch and Chemical Company, Thailand.

Coating solution preparation
For pure clove oil coating solution, clove oil was diluted with (30µl/ml) dimethylsulphoxide (DMSO) (Sigma-Aldrich Laborchemikalien GmbH, Germany) in a series of half-fold dilutions, 5.0000%, 2.5000%, 1.2500%, 0.625% and 0.3125% (v/v). In addition, for clove oil in hydrophobic starch matrixes, the suspension of hydrophobic starch at 5% and 8% w/w (dry weight basis) was heated using hot plate stirrer (Stuart Scientific, UK) at 95 ± 5˚C until the solution formed gel and then the temperature of the solution was decreased and kept constant at 65 ± 5˚C using a water bath (Memmert, Germany). Following this, clove oil was added into starch solutions in various concentrations as follows: 5.0000%, 2.5000%, 1.2500%, 0.6250% and 0.3125% (w/w). The mixtures were then homogenized for one minute. The homogenized procedure made the hydrophobic starch and clove oil mix well and have uniform suspension.

Determination of minimum inhibitory concentration (MIC) of clove oil and coating solutions
The MIC of clove oil and clove oil in starch matrix was determined by the agar well diffusion technique [23]. Each bacterium was first sub-cultured in nutrient broth at 37˚C for 24 hours. A thousand microlitres (1000 µl) of standardized inoculum (10⁶ CFU/ml) of each test bacterium was inoculated in a Petri dish (Griener, Germany) using pour plate technique. The plates were allowed to dry and a sterile cork borer (5 mm. diameter) was used to bore wells in the agar. A 40 µl volume of each dilution of solutions was added aseptically into the wells of nutrient agar. DMSO without clove oil, 5% or 8% hydrophobic starch solutions were used as negative control.
The agar plates were incubated at 37°C for 18-24 hours. 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 clove oil solutions were detected as clear zones around the wells and diameters of clear zones were expressed in millimeters (mm). Only clear zones that larger than 7 mm were recorded [24]. Minimum inhibitory concentrations (MICs) which were defined as lowest concentrations of a particular clove oil solution that can inhibit certain bacteria were also investigated.

**Results and Discussion**

Pure clove oil in DMSO was the most effective antimicrobial coating solution against both gram-negative and gram-positive bacteria, especially for *Escherichia coli*, that showed a minimum inhibitory concentration (MIC) at 1.25%, while the MIC for the other two bacteria (*Bacillus cereus* and *Staphylococcus aureus*) was at 2.5% (Fig. 1(a)). For this study, DMSO was intended to be used as a solvent for clove oil. Previous studies have indicated that concentration levels of DMSO solution that can inhibit antimicrobial growth were far higher than the concentration levels employed in this study [25]. As a result, MICs observed were influenced entirely by clove oil. This issue can be confirmed by the negative controls composed of DMSO without clove oil, 5% and 8% hydrophobic starch that showed no inhibitory effect for all bacteria. Pure clove oil solution may be able to release more active essential oil (EO) without any blocking of starch compound. However, the amount of released oil vapour versus time of the pure clove oil system compared to those of starch matrix system was out of the scope of this study. For pure clove oil, it was also possible that the oil vapour may release rapidly at the initial stage [26, 27] resulting in more effective antimicrobial results.

Results of clove oil suspension in hydrophobic starch matrix revealed that a coating solution containing clove oil in 8% hydrophobic starch matrix can better inhibit *Bacillus cereus* and *Staphylococcus aureus* compared to clove oil in 5% hydrophobic starch matrix (Fig. 1(b, c)). The MICs of clove oil in 8% hydrophobic starch matrix were found to be similar to pure clove oil in DMSO solution. However, slightly smaller clear zones for all bacteria were found for clove oil in the starch matrix. On the other hand, clove oil in 5% hydrophobic starch matrix was not able to inhibit *Bacillus cereus* and *Staphylococcus aureus*, whilst it was able to inhibit *Escherichia coli* with the MIC at 2.5% (Fig. 1(b)). According to the study of Tepsorn, [22], various Thai medicinal extracts were not able to reduce the numbers of *Bacillus cereus* in the presence of interfering substances and carbohydrates and do not appear to protect bacteria from the action of medicinal plant extracts as much as fat and protein do.

Normally, native starches lack active surface properties and have to be chemically modified or used in conjunction with emulsifying agents in order to encapsulate hydrophobic products such as aroma compounds. For instance, hydrophobic octenyl succinate anhydrous (OSA) groups can be grafted to native or hydrolyzed starches to impart their emulsifying abilities such as those found in hydrophobic starch under this study. The presence of hydrophobic groups contributes to the absorption of oil into the matrix and also increases the oil loading during the encapsulation process [20]. Thus, hydrophobic starch matrix acts as a reservoir for clove oil and also decreases the separation between oil and water in the coating solutions. Therefore, the higher concentration of hydrophobic starch in the coating solution, the better the antimicrobial inhibitory effect for the coating solution.
Figure 1. The antimicrobial activity of clove oil at various concentrations (a) pure clove oil (b) clove oil in 5% hydrophobic starch matrix (c) clove oil in 8% hydrophobic starch matrix.
Figure 2. Inhibitory zones of (a) pure clove oil (b) clove oil in 5% hydrophobic starch matrix (c) clove oil in 8% hydrophobic starch matrix against *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus*.

Appendini and Hotchkiss [28], mentioned in their study that carriers or matrixes containing antimicrobial agent coating on the packaging materials can release antimicrobial substances onto the surface of the food. The system is more effective than direct application of antimicrobial agents on the food itself, because it slows the migration of the agents away from the surface and thus helps to maintain high concentrations where needed.
Conclusion

Pure clove oil revealed better antimicrobial inhibition than clove oil in hydrophobic starch matrixes. However, rapid release of oil vapour may be observed for pure clove oil solution compared to oil in starch matrixes. An important finding of this study was that starch concentration levels showed significant impact on the antimicrobial activities of the coating solutions. Finally, clove oil of sufficient concentration in hydrophobic starch matrixes proved to be successful in inhibiting all bacteria under the study.

References


