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Effect of Antimicrobial Extracts on the Characteristics of Tapioca-Based Edible Coating for Coated Tomatoes

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A B S T R A C T

Edible coating is a food coating material that can reduce spoilage due to transpiration and respiration. However, coating alone is not having any effect, especially for high-water-content fruits, which can be susceptible to bacterial decay. The addition of antimicrobial extracs may inhibit the growth of spoilage bacteria. This research aims to determine the minimum concentration of antimicrobial extract needed to extend the shelf life and reduce weight loss of tomatoes, identify the dominant bacteria causing fruit decay (Escherichia coli or Staphylococcus aureus), and determine the most effective antimicrobial extract in preventing microbial growth. The study began with the extraction process, testing the active compounds in the extracts, combining edible coating with extracts (white turmeric and bitter melon at concentrations of 0%, 2.5%, 5%, 10%, 20%, 30%, and 100%), and coating the tomatoes. Tests included shelf life, weight loss, the best antimicrobial extract, and inhibition zone diameter. Increasing the extract concentration resulted in a longer shelf life of up to 14 days and reduced weight loss to 3.53%. The antimicrobial extracts contain saponins, steroids, and triterpenoids. The minimum concentration of bitter melon and white turmeric extract to inhibit bacterial activity was 30%, providing an inhibition zone diameter ranging from 10-12 mm, indicating moderate efficacy. The dominant bacterium in the decay of tomato fruit is Staphylococcus aureus.

INTRODUCTION

Edible coatings have been extensively researched as alternatives to wax coatings to preserve the quality of perishable fruits and vegetables. The main causes of decay are attributed to high transpiration rates and post-harvest diseases. Various materials can be utilized for edible coatings, including those based on polysaccharides, proteins, fats, and composites. These coatings serve as a protective layer, addressing issues related to moisture loss and susceptibility to post-harvest diseases, thereby extending the shelf life of fruits and vegetables. This research explores the potential of edible coatings as an effective solution in maintaining the freshness and quality of produce, offering a promising avenue for sustainable post-harvest preservation.

Tomatoes, with a water content of 94%, are particularly prone to decay [1][2]. The application of edible coating significantly enhances the firmness of tomatoes, reduces weight loss, and decreases the respiratory rate. Additionally, the coating process can effectively slow down changes in shape [3][4].

However, the application of coating alone is not entirely effective in preventing decay. Decay processes can occur more rapidly when tomatoes are infected with spoilage bacteria such as Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), and Salmonella. [5][6]. The addition of white turmeric to the edible coating can prevent food spoilage for up to the 15th day [7]. The utilization of white turmeric extract at a concentration of 100 μL can result in a diameter (indicating resistance to E. coli bacteria) of approximately 5.84 mm [8]. White turmeric extract at a concentration of 20% (w/v) has been proven effective in preventing S. aureus bacteria and forming a clear zone with a diameter of 13.6 mm [9]. Furthermore, the use of bitter melon extract also demonstrates resistance against E. coli and forms a diameter of 14 mm [10].

The effectiveness of an antimicrobial can also be determined by the minimum inhibitory concentration (MIC). MIC is the lowest concentration required to prevent the proliferation of microbes, as indicated by the increasing clarity of the bacterial test solution [10]. The use of bitter melon extract at a concentration of 2.5% against E. coli bacteria in vitro is characterized by the absence of turbidity in the solution containing bitter melon extract [11].

Despite the positive impact of edible coatings on enhancing the quality and shelf life of tomatoes, their efficacy in preventing decay, particularly when tomatoes are infected with spoilage bacteria, remains a challenge. This study aims to address this limitation by exploring additional strategies, such as the incorporation of antimicrobial extracts like white turmeric and bitter melon, to enhance the protective properties of edible coatings and prolong the post-harvest preservation of tomatoes.

Despite the positive impact of edible coatings on enhancing the quality and shelf life of tomatoes, their effectiveness in preventing decay, especially when tomatoes are infected with spoilage bacteria, remains a challenge. This study aims to address this limitation by exploring additional strategies, such as incorporating antimicrobial extracts like white turmeric and bitter melon, to enhance the protective properties of edible coatings and extend the post-harvest preservation of tomatoes. The research investigates the combination of antimicrobial extracts with edible coatings to assess their ability to extend the shelf life of tomatoes. Additionally, the study aims to compare the effectiveness of white turmeric and bitter melon extracts as antimicrobials at the same concentration in preventing the proliferation of E. coli and S. aureus.

METHODS

The equipment and materials employed in this study included chemical beakers, a Bunsen burner, Erlenmeyer flasks, a hotplate, magnetic stirrer, micropipette, volumetric flask, rotary evaporator, graduated pipette, petri dish, needle, test tube, analytical balance, UV-VIS spectrophotometer, incubator, autoclave, and shaker. The substances utilized encompassed tomatoes, tapioca flour, glycerol, carboxymethyl cellulose (CMC), distilled water (Aquadest), Mueller-Hinton Agar (MHA), ethanol, bitter melon powder, turmeric powder, nutrient agar, sodium chloride (NaCl), hydrochloric acid (HCl 37%, HCl 1 N), ferric chloride ($FeCl_3$), chloroform ($CHCl_3$), acetic anhydride, sulfuric acid (H_2SO_4 95%), Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), nutrient broth, and filter paper.

The production of antimicrobial extracts from white turmeric and bitter melon

The method involved the application of the maceration method, followed by concentration using a rotary evaporator. Antimicrobial extracts from white turmeric and bitter melon were obtained by dissolving 200 grams each of their respective powder extracts in 1 liter of food-grade 96% ethanol. The solution was then shaken for 30 minutes in a shaker. Once homogenized, the solution was allowed to settle for 12 hours until two layers formed. The upper layer was then taken and evaporated using a rotary evaporator to yield a more concentrated extract.

Testing the Antimicrobial Activity of White Turmeric and Bitter Melon Compounds

The testing of flavonoid content involved mixing 0.5 grams of extract with Mg powder in 1-2 ml of hot water, concentrated hydrochloric acid (HCl) in 4-5 ml, and ethanol in 4-5 ml. The occurrence of a color change to red-yellow-orange confirms the presence of flavonoids in the extract [11].

The assessment of tannin content was conducted by mixing 0.5 grams of the extract with 2 drops of iron (III) chloride. The occurrence of a color change to green-blue confirms the presence of tannins in the extract. Simultaneously, the evaluation of saponin content involved combining 0.5 grams of the extract with 0.5 ml of hot water and adding 2 drops of hydrochloric acid (HCl). The presence of stable foam indicates the extract contains saponins.

The assessment of triterpenoid and alkaloid content involved contacting 0.5 grams of the extract with 0.5 ml of chloroform, 0.5 ml of acetic acid, and 1-2 ml of sulfuric acid. The occurrence of a color change to purple-red confirms the presence of triterpenoids in the extract, while a green color formation indicates the presence of steroids [12].

Edible Coating Formulation

Edible coating is created using tapioca flour with the addition of glycerol as a plasticizer, followed by the incorporation of antimicrobial extracts from white turmeric and bitter melon at various concentrations. The basic formula for the edible coating comprises 4 g of tapioca flour, 100 ml of distilled water, 1.75 ml of glycerol, 0.5 g of carboxymethyl cellulose (CMC), and the respective extracts from white turmeric and bitter melon [7]. After thorough mixing, the solution is allowed to stand for 2 hours before being applied to the tomatoes [13].

Testing the Clear Zone Diameter

The analysis was conducted using both the disc diffusion and well methods. In the disc diffusion method, paper discs were immersed in the extract solution, and then the discs were placed on the surface of agar medium that had been inoculated with bacteria. Meanwhile, the well method involved creating holes on the agar surface that had been inoculated with bacteria, followed by the addition of the antimicrobial extract with predetermined concentrations to each well. The wells were positioned with 2 cm from the dish's wall and 2 cm apart from each other, with an agar depth of approximately 4 mm.

Minimum Inhibitory Concentration (MIC) Testing

The process of testing the minimum inhibitory concentration against Escherichia coli and Staphylococcus aureus involved preparing extract solutions at concentrations of 0%, 2.5%, 5%, 10%, 20%, 30%, and 100%, which were subsequently diluted at a 1:2 (v/v) ratio. The testing was then conducted by measuring the absorbance values of the solutions using a UV-Visible Spectrophotometer. A wavelength of 625 nm was employed for the measurements. Absorbance measurements were taken both before and after the incubation of the solutions. An increase in absorbance values indicated that bacteria could still proliferate, and the concentration of the extract used was ineffective in inhibiting bacterial growth.

Weight Loss Analysis

The analysis was conducted by periodic weighing over a 14-day period, followed by the calculation of weight loss using equation (1).

$$Weight\ Loss = \frac{Initial\ weight - Final\ weight}{Initial\ weight}\ x\ 100\% \quad \dots (1)$$

Shelf-Life Assessment

Shelf-life is estimated through the observation of color and physical characteristics of tomatoes over a predetermined period. The color and physical properties of tomatoes with and without edible coating are also compared. The observation period is set for 14 days.

RESULT AND DISCUSSION

Weight Loss Analysis Result

One external quality parameter of tomatoes is their weight loss. The influence of the concentrations of antimicrobial extracts from bitter melon and white turmeric powders on their respective weight loss can be observed in **Figure 1** and **Figure 2**. The codes K and P indicate the types of extracts used, namely white turmeric (K) and bitter melon (P). The digits alongside the K and P codes represent the percentage concentration of the extracts used. The blank represents a sample of tomatoes that were not coated with edible coating.

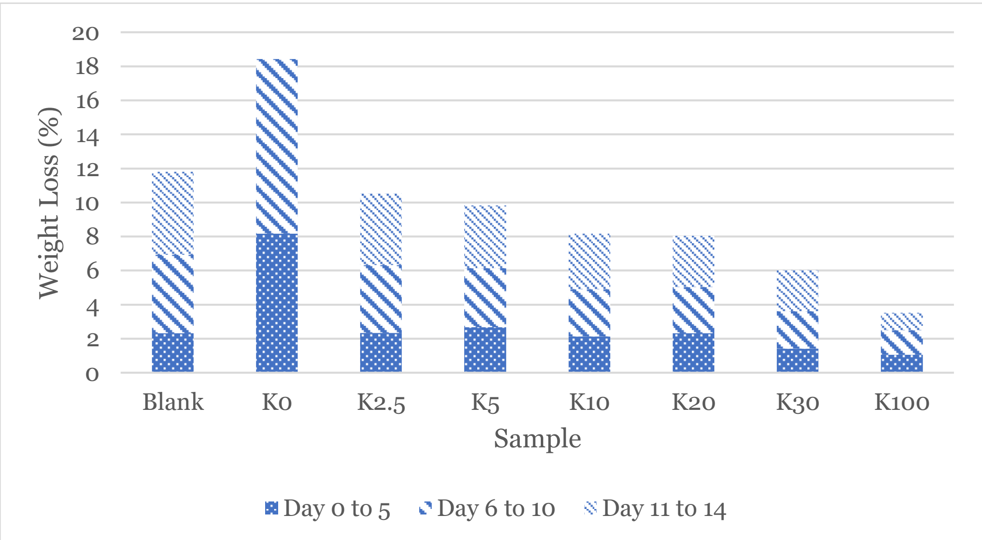

Figure 1. The Impact of White Turmeric Antimicrobial Extract Concentration Variations on Weight

Loss

In Figure 1, it is evident that the increase in antimicrobial concentration in the edible coating results in a decrease in weight loss. The highest antimicrobial concentration (100%; K100) yields the lowest total weight loss at 3.25%. The K0 sample (tomato coated with edible coating without antimicrobial extract) exhibits the highest weight loss value, reaching 18.43%. This proves that the addition of antimicrobial extracts can maintain the tomato weight loss to only 3.25% over the 14-day period. The edible coating supplemented with white turmeric extract can inhibit the exchange of oxygen and water vapor in and out of the tomato, slowing down fruit respiration and causing the fruit to decay more slowly.

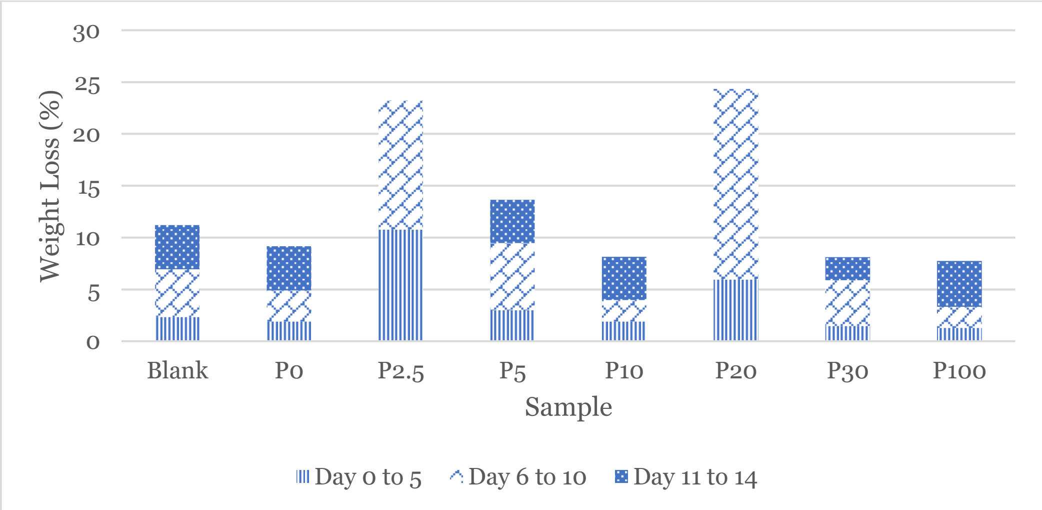

Figure 2. The Impact of Varied Concentrations of Bitter Melon Antimicrobial Extract on Weight Loss

In **Figure 2**, it is evident that the P20 sample exhibits the highest total weight loss, reaching 24.33%, within the first 10 days of storage. The lowest total weight loss is observed in the P100 sample (tomato coated with edible coating and supplemented with 100% concentration of bitter melon), maintaining at 6.55% until day 14 of storage. On day 14, the tomatoes in the P100 sample still exhibit a fresh physical appearance. Tomatoes that exhibited signs of decay before day 14 were the P2.5 sample, with a storage period only up to day 6, and the P20 sample, with a storage period only up to day 10. This occurrence is attributed to the different levels of maturity in the tomatoes used. The increased addition of bitter melon antimicrobial to the edible coating proves effective in inhibiting the processes of respiration and transpiration.

Results of Secondary Metabolite Compound Testing

Phytochemical testing was conducted on white turmeric and bitter melon extracts to confirm the presence of active compounds such as flavonoids, triterpenoids, saponins, tannins, and steroids. The results of these tests are presented in **Tables 1** and **2**, providing crucial insights into the chemical composition of both extract types. The identification of these compounds is a key step in understanding the potential antimicrobial activity of white turmeric and bitter melon extracts, serving as a foundation for further exploration related to the utilization of these compounds in food coating applications.

Table 1. Results of Active Compound Testing in White Turmeric Extract

Number	Active Compound	Reagent	White Turmeric Extract
1	Flavonoid	HCl 37% and Mg powder	-
2	Tanin	$FeCl_3$ 1%	-
3	Saponin	Aquadest and HCl 1N	+++
4	Triterpenoid	$CHCl_3$ and H_2SO_4	+
5	Steroid	Acetic anhydride and H_2SO_4	+++

\+ Contains active compounds.
\- Does not contain active compounds

The quantity of symbols indicates an increasing content.

Table 1. Results of Active Compound Testing in Bitter Melon Extract

Number	Active Compound	Reagent	Bitter Melon Extract
1	Flavonoid	HCl 37% and Mg powder	-
2	Tanin	$FeCl_3$ 1%	-
3	Saponin	Aquadest and HCl 1N	+++
4	Triterpenoid	$CHCl_3$ and H_2SO_4	+++
5	Steroid	Acetic anhydride and H_2SO_4	-

\+ Contains active compounds.
\- Does not contain active compounds
The quantity of symbols indicates an increasing content.

The results of phytochemical compound testing reveal that white turmeric extract contains saponins, triterpenoids, and steroids, while bitter melon extract contains saponins and triterpenoids. Saponins could inhibit bacterial growth by disrupting the cytoplasmic membrane, whereas triterpenoids and steroids hinder bacterial growth by disintegrating the cell membrane. This information underscores the potential antimicrobial properties of these compounds and provides valuable insights into their mechanisms of action against bacteria.

Results of Clear Zone Diameter and Minimum Inhibitory Concentration Testing

The agar surface is coated with the test bacteria solution and dried with its extract, followed by incubation for 2 hours at 37 °C. The formation of clear zones is observed around the wells. Compounds with inhibition zones ≤ 10 mm exhibit weak inhibitory response, 10-15 mm are classified as moderate, 16-20 mm are considered strong, and ≥ 20 mm are categorized as very strong inhibition [14]. The test results are depicted in **Figures 3** to **6**.

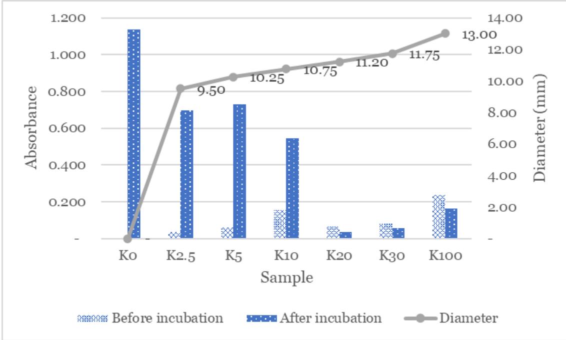

Figure 3. Test Data for Clear Zone Diameter and Minimum Inhibitory Concentration of White Turmeric Extract Against E. coli Bacteria

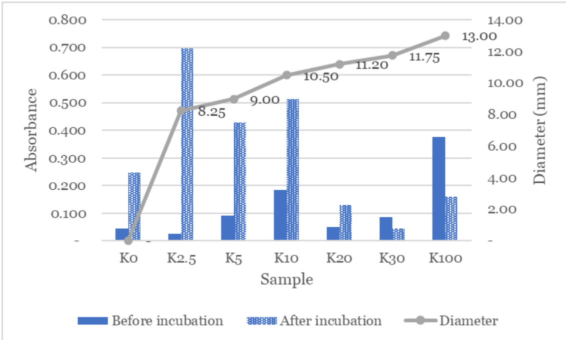

Figure 4. Test Data on Clear Zone Diameter and Minimum Inhibitory Concentration of White Turmeric Extract Against S. aureus Bacteria"

Figures 3 and **4** illustrate the influence of white turmeric extract concentration in inhibiting E. coli and S. aureus bacteria. At a concentration of 2.5%, the inhibitory response against E. coli is weak, with a diameter of <10 mm. At concentrations ranging from 5% to 100%, clear zones with diameters of 11-15 mm are formed, indicating a moderate inhibitory capability. White turmeric extract's response to S. aureus at concentrations of 2.5%-10% results in a diameter of <10 mm, categorizing it as weak. At concentrations of 20%-100%, white turmeric extract is classified as a moderate antimicrobial with clear zone diameters ranging from 11-15 mm. The minimum inhibitory concentration (MIC) of white turmeric to inhibit E. coli is 20%, while for S. aureus, it is 30%.

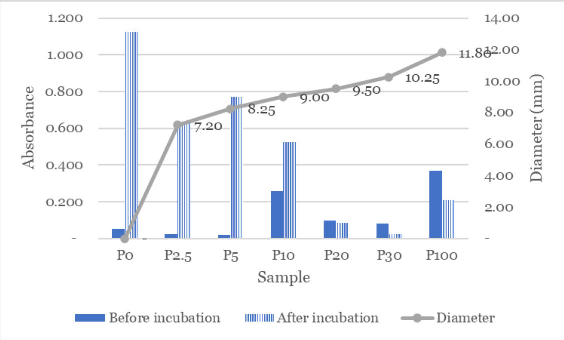

Figure 5. Test Data on Clear Zone Diameter and Minimum Inhibitory Concentration of Bitter Melon Extract Against E. coli Bacteria

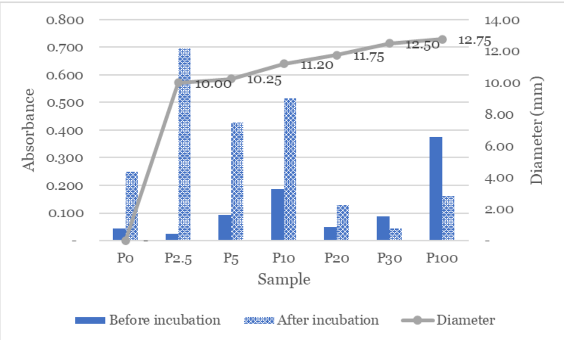

Figure 6. Test Data on Clear Zone Diameter and Minimum Inhibitory Concentration of Bitter Melon Extract Against S. aureus Bacteria

An increase in absorbance values indicated that bacteria could still proliferate, and the concentration of the extract used was ineffective in inhibiting bacterial growth. At concentrations ranging from 2.5% to 20%, the ability of bitter melon extract to inhibit the growth of S. aureus bacteria was considered weak, with a clear zone diameter of <10 mm. However, at concentrations of 30% and 100%, its antimicrobial ability was moderate, resulting in a diameter of 11-15 mm. The response of bitter melon extract to S. aureus bacteria at concentrations from 2.5% to 100% formed clear zones with a diameter of 10-15 mm, indicating a moderate effectiveness in inhibiting bacterial growth.

Based on Figures 3 to 6, the minimum concentration required for white turmeric and bitter melon extracts to inhibit Escherichia coli bacteria is 20%, while for Staphylococcus aureus bacteria, it is 30%. At a concentration of 20%, the coating contains 200 ppm of the extract, and at 30%, it contains 300 ppm of the extract. Antimicrobial activity at MIC of 100-500 ppm is considered moderate [15].

CONCLUSION

In conclusion, this study investigated the efficacy of antimicrobial extracts from bitter melon and white turmeric in edible coatings for preserving tomato quality. Both extracts demonstrated promising results, with white turmeric, particularly at a concentration of 100%, exhibiting the lowest total weight loss of 3.25% over 14 days. The presence of active compounds, such as saponins and triterpenoids, in these extracts was confirmed, emphasizing their potential for broader applications. Antimicrobial testing revealed moderate inhibitory activity against E. coli and S. aureus, with an MIC of 20% for white turmeric and 30% for bitter melon. This comprehensive approach offers valuable insights into the potential of these natural antimicrobials for enhancing food preservation and quality, paving the way for further research in optimizing formulations and extending applications across various food products.

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