BIOACTIVE COATINGS WITH HARZIANIC ACID: A PROMISING STRATEGY FOR ENHANCING MICROBIOLOGICAL CONTROL IN TOMATOES

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ABSTRACT

In this study, harzianic acid (HA) was produced by *Trichoderma harzianum* using cocoa pod husk extract as a substrate. HA was incorporated into a hydrocolloid-based coating formulation, consisting of equal proportions of guar gum, pectin, and carboxymethylcellulose (1 %, w/v), glycerol (5 g/L), and an HA nanoemulsion (0.25 g/L), which were applied to 80 unripe green tomatoes for 21 days at 25°C (70% RH). The bioactive coating proved effective in reducing contamination by fungi, mesophilic bacteria, total coliforms, and coliforms at 45°C during 21 days of storage at 25°C. Sensory analysis based on consumer perception indicated improvements in the color and firmness of coated tomatoes, resulting in increased purchase intention among consumers. The bioactive coatings demonstrated efficiency in microbiological control, highlighting its potential in post-harvest quality preservation and food safety, offering a promising strategy for agricultural products. However, further studies are crucial to address the safety of HA use in food products.

Keywords: Cocoa pod husk, *T. harzianum*, Bioactive film, Post-harvest, Quality preservation.

1 INTRODUCTION

Tomato (*Solanum lycopersicum* L.), a globally cultivated agricultural commodity, faces challenges due to its high water content and vulnerability to mechanical damage, resulting in a limited post-harvest lifespan and significant losses.[1] Fungal infections, especially those caused by *Botrytis cinerea*, *Alternaria solani*, and *Fusarium oxysporum*, play a substantial role in contributing to post-harvest losses in tomatoes.[2] Edible coatings, extensively researched for their efficacy in preserving fruit quality, especially in addressing lipid oxidation and enzymatic/microbial activities, commonly utilize hydrocolloids such as guar gum, pectin, and carboxymethylcellulose (CMC) due to their abundance and versatile applications.[3,4] Despite the promising nature of edible coatings, additional research is required to investigate the controlled release of bioactive compounds within these coatings. In this context, harzianic acid (HA), a secondary metabolite from *Trichoderma harzianum*, emerges as a potential biocontrol agent against fungi such as *Fusarium oxysporum* in tomatoes, owing to its antimicrobial properties.[5,6] HA demonstrates antimicrobial activity against Gram-positive bacteria and methicillin-resistant strains.[7] Its mechanism of action involves the inhibition of acetohydroxyacid synthase, a key enzyme in the biosynthetic pathway of branched-chain amino acids.[8] This study aims to assess the impact of incorporating HA into bioactive coatings formulated with polymers such as guar gum, CMC, and pectin on the microbiological control of tomatoes shelf life during 21 days of storage at 25°C (70 % relative humidity) were evaluated.

2 MATERIAL & METHODS

The cocoa pod husk (CPH) was dried in a circulating oven at 60 °C for 4 days. Following drying, the CPH was ground using a knife mill and sieved to obtain particles with a diameter smaller than 16 mesh (<1 mm). An extract from CPH was prepared at a concentration of 40g/L for HA production. This extract was supplemented with 0.5% (w/v) potato dextrose broth and sterilized at 121°C for 30 min. The liquid fraction (100 mL) was inoculated with 1.0×10^5 spores/mL of *T. harzianum*, and cultivation occurred in a greenhouse at 25°C for 25 days. HA extraction and purification were conducted according to the method described in the literature.[9] For the preparation of HA, 80 g of distilled water, 10 g of HA/soybean oil (in a 2:8 w/w ratio), and 10 g of Tween 80 were emulsified using an Ultra Turrax disperser (Tecnal, TE-147) at 12,000 rpm for 30 min. The bioactive coating was formulated using guar gum (1%, w/v), CMC (1%, w/v), and citrus pectin (1%, w/v), each solubilized separately at 70°C with 800 rpm. After cooling, glycerol (5 g/L) was added under agitation (800 rpm), and the pH was adjusted to 4.0 (using NaOH or HCl, 0.1N). The final bioactive coating comprised 1% (w/v) of a polymeric mixture of guar gum, citrus pectin, and CMC (33.3% v/v/v each), glycerol (5 g/L), and HA at 0.25 g/L.

A total of 170 tomato fruits were randomly divided into two groups: an uncoated group (UC) and a coated group (C). Unripe green tomatoes were surface disinfected with a 10 % (v/v) sodium hypochlorite solution (2.5 % active chlorine) for 15 min. and air-dried at room temperature. UC tomatoes were immersed in distilled water for 1 min., while C tomatoes were immersed in the bioactive coating solution for the same duration. After the immersion process, all fruits were air-dried at room temperature and stored in a controlled environment at 25ºC with 70 % RH for 21 days.

At different storage times (0, 5, 10, 15, and 21 days), five tomato fruits were homogenized, and microbial analyses were conducted. Total bacteria, molds and yeasts were analyzed using the total plate count method, while total coliforms, thermotolerant coliforms, and *E. coli* were assessed using the Most Probable Number (MPN) method. *Staphylococcus aureus* was counted using the direct plate count method (coagulase-positive *S. aureus*), following American Public Health Association recommendations. *Salmonella* spp. analysis followed the ISO 6579:2007 method according International Organization for Standardization recommendations.[10]

Affective acceptance and purchase intention tests involved 30 evaluators, encompassing both men and women aged between 20 and 65. During the assessment, participants visually evaluated the color, shine, overall appearance, and finger firmness of tomatoes, comparing coated (C) and uncoated (UC) samples. This evaluation took place on the 13th day of storage, with six fruits in each group. Evaluations were made on an 11-point category scale, ranging from 1 (extremely unpleasant) to 11 (extremely pleasant). For the purchase intention assessment, evaluators rated the tomatoes on a 5-point category scale, ranging from "definitely would not buy" to "definitely would buy," considering them as fresh fruits ready for consumption. The experiments were conducted in triplicate, unless otherwise specified, and expressed as mean ± standard deviation. Graphs were generated in Excel® (Office 2019).

3 RESULTS & DISCUSSION

Throughout the 21-day storage period for tomatoes, no presence of *Salmonella* spp., *Escherichia coli*, or *Staphylococcus aureus* was detected in both groups UC and C. However, significant variations were observed in the concentrations of molds and yeasts, total mesophilic bacteria (TMB), total coliforms, and coliforms at 45°C over time (Figure 1 and Table 1). Regarding molds and yeasts, the initial concentration in group UC was 4.11±0.11 log CFU/g, reaching a peak on the tenth day at 9.27±0.22 log CFU/g, and subsequently decreasing by 2 log CFU/g until the twenty-first day of storage. Interestingly, the C fruits maintained molds and yeasts at 4 log CFU/g until the 10th day of storage, after which there was a complete decrease in concentration (Figure. 1A). The decrease in the concentration of molds and yeasts can be attributed to the antimicrobial and antifungal properties of HA, as reported in previous studies.[11]

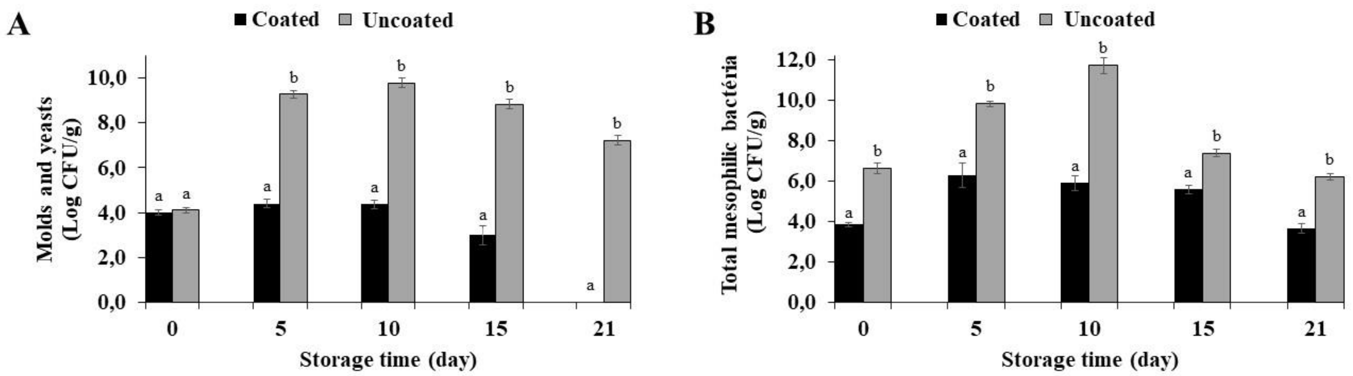

Figure 1. Influence of bioactive coating containing harzianic acid on tomatoes stored at 25ºC (A) Molds and yeasts, (B) Total mesophilic bacteria. Different letters indicate significant differences ($p<0.05$) for the same storage time.

The initial concentration of TMB was 3.84±0.12 log CFU/g in fruits coated with HA and 6.62±0.25 log CFU/g in UC fruits from group UC (Figure 1B). Significant differences between groups C and UC were observed throughout the 21-day storage period. Interestingly, an increase in TMB contamination was observed in both group C (up to 6.28±0.59 log CFU/g) and group UC (up to 11.72±0.38 log CFU/g), but by the end of storage period, both groups returned to the initial concentration. In a study[4] involving the incorporation of 2 % cardamom essential oil in CMC, a significant reduction in the concentration of TMB compared to the control was observed during the 15 days of tomato storage at 25±2°C and 60 % RH. Additionally, a previous study[12] utilizing 0.2 % ethanolic extract of coriander seeds in 1.5 % guar gum significantly reduced the concentration of TMB in tomatoes stored at 10ºC for 60 days. Tomatoes coated only with 1.5 % guar gum were insufficient to reduce the concentration of TMB, molds and yeasts when compared to uncoated fruits.[13] These findings highlight the significance of adopting strategies to manage microbial contamination in tomatoes during the post-harvest, and the utilization of bioactive coatings is emerging as a practical and effective quality assurance strategy.

The results of total coliforms and coliforms at 45ºC for tomatoes treated with the HA bioactive coating over 21-day storage period are presented in Table 1.

Table 1: Influence of bioactive coating containing harzianic acid on tomatoes stored at 25ºC on total coliforms and coliforms at 45ºC

Storage time (day)	Total coliforms (MPN/g)		Coliforms at 45°C (MPN/g)	
	Coated	Uncoated	Coated	Uncoated
0	93	>1,100	3	36
5	36	1,100	3	36
10	43	>1,100	3,6	75
15	43	1,100	27	>1,100
21	72	>1,100	43	1,100

Notably, the aseptic process conducted on the tomatoes before the experiment did not effectively reduce the microbiological load of total coliforms (>1,100 MPN/g) and coliforms at 45ºC (36 MPN/g) for the UC group. However, the initial outcomes for tomatoes coated with HA demonstrated an immediate and effective action of the developed bioactive coating. The values for total coliforms (93 MPN/g) and coliforms at 45ºC (3 MPN/g) were drastically reduced. After 15 days of storage, the UC group exhibited coliforms at 45ºC levels exceeding 1,100 MPN/g, indicating a loss of microbiological safety for fresh tomato consumption. In contrast, the fruits coated with HA maintained food safety, with the coliforms at 45ºC value remaining at 43 MPN/g. A previous study[4] incorporating 2 % cardamom essential oil into CMC significantly reduced the concentration of *E. coli* in tomato fruits during 15-day storage at 25ºC (60% RH). However, in another study,[14] both UC and C tomatoes showed a coliforms at 45ºC count of <3.0 MPN/g. The microbiological results in tomato fruits revealed that the bioactive coating was effective in reducing contamination by molds, yeasts, mesophilic bacteria, total coliforms, and coliforms at 45ºC over the 21-day storage period at 25ºC. This study underscores the potential of the developed coating as a strategy to enhance the quality and food safety of tomatoes. However, studies are crucial to address the safety of HA use in food products.

The results of the sensory analysis of tomato fruits conducted on the 13th day of storage are presented in Figure 2.

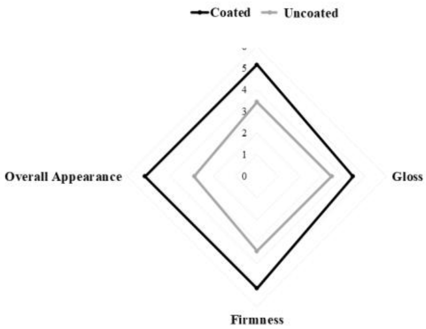

Figure 2. Affective sensory analysis of tomatoes coated or not with harzianic acid bioactive coating in terms of gloss, color, firmness, and overall appearance.

Four parameters - color, gloss, finger touch firmness, and overall appearance – were assessed using six C fruits and six UC fruits. Notable differences were observed in color and fruit firmness between the groups, indicating that the HA coating had a positive impact on the coloration of the tomato fruits. However, gloss and overall appearance exhibited no significant differences between the two groups, suggesting that the coating had no discernible impact in these aspects. These findings highlight the potential of coating tomatoes with HA to enhance the color and firmness of the fruits. The results from the purchase intention analysis further revealed that 48.39 % of the participants expressed a definite willingness to purchase tomatoes coated with HA, while 22.58 % expressed the same intention for the UC group. Additionally, 41.94 % of the participants indicated that they would definitely or probably buy the coated fruits, whereas 32.26 % expressed the same intention for the UC group. These results underscore a higher acceptance and willingness of consumers to acquire the coated fruits compared to the UC group. This preference may be associated with perceived benefits, such as the improvement in color and firmness of the fruits provided by the HA coating. However, it is important to note that further research is necessary to assess the coating's effects at different storage stages and consider consumer preferences regarding the sensory attributes of the fruits.

4 CONCLUSION

The formulated coating not only showed efficacy in microbiological control but also demonstrated the ability to enhance the color and firmness of the fruits. These results underscore the significance of HA as an effective disease control agent in tomatoes, emphasizing the potential of the developed formulation in preserving post-harvest quality. This dual impact contributes not only to food safety but also enhances the overall value of agricultural products. However, further studies are needed on the safety of HA use in food products, as well as its impact on human health.

REFERENCES

1 Donjio, R.T., Nguemezi, J.A., Anoumaa, M., Phounzong, E.T., Kenfack, J.O., Fonkou, T. 2023. J. Food Qual. 1019310.
2 Wahome, P.K. 2019. UNISWA J. Agric. 20. 34–40.
3 Aguilar-Veloz, L.M., Calderón-Santoyo, M., Carvajal-Millan, E., Martínez-Robinson, K., Ragazzo-Sánchez, J.A. 2022. LWT-Food Sci. Technol. 156. 113022.
4 Das, S.K., Vishakha, K., Das, S., Chakraborty, D., Ganguli, A. 2022. Biocatal. Agric. Biotechnol. 42. 102369.
5 Nurbailis, Djamaan, A., Rahma, H., Liswarni, Y. 2019. Biodiversitas. 20 (10). 2915–2920.
6 Vinale, F., Flematti, G., Sivasithamparam, K., Lorito, M., Marra, R., Skelton, B.W., Ghisalberti, E. L. 2009. J. Nat. Prod. 72 (11). 2032–2035.
7 Filippis, A., Nocera, F. P., Tafuri, S., Ciani, F., Staropoli, A., Comite, E., Bottiglieri, A., Gioia, L., Lorito, M., Woo, S.L., Vinale, F., De Martino, L. 2021. Nat. Prod. Res. 35 (23). 5440–5445.
8 Xie, L., Zang, X., Cheng, W., Zhang, Z., Zhou, J., Chen, M., Tang, Y. 2021. J. Am. Chem. Soc. 143 (25). 9575–9584.
9 Pang, G., Sun, T., Ding, M., Li, J., Zhao, Z., Shen, Q., Cai, F.M. 2022. J. Agric. Food. Chem. 70 (37). 11769–11781.
10 Silva, N., Junqueira, V.C.A., Silveira, N.F.A., Gomes, R.A.R., Okazaki, M.M. 2017. Manual de Métodos de Análise Microbiológica de Alimentos e Água, 5th ed. Blucher, São Paulo.
11 Ouyang, X., Hoeksma, J., Beenker, W.A.G., Beek, S., Van der, Hertog, J. den. 2021. BioRxiv. 2021.12.03.471066.
12 Naeem, A., Abbas, T., Ali, T.M., Hasnain, A. 2018. J. Food Meas. Charact. 12. 2725–2734.
13 Ruelas-Chacon, X., Contreras-Esquivel, J.C., Montañez, J., Aguilera-Carbo, A.F., Reyes-Vega, M.L., Peralta-Rodriguez, R.D., Sanchéz-Brambila, G. 2017. J. Food. Qual. 8608304.
14 Araújo, J.M.S., Siqueira, A.C.P., Blank, A.F., Narain, N., Santana, L.C.L.A. 2018. Food Bioproc. Tech. 11. 1750–1760.