

Quality Attributes of Fresh Poultry Meat Wrapped in Corn starch-based Films Incorporated with Nanocellulose Fiber and Thymol for Active Packaging Application

Norhazirah Nordin¹, Siti Hajar Othman^{1,2,*}, Roseliza Kadir Basha¹, Suraya Abdul Rashid²

¹*Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, Serdang, Malaysia*

²*Institute of Nanoscience and Nanotechnology, Universiti Putra Malaysia, Serdang, Malaysia*

**Corresponding author's E-mail: s.hajar@upm.edu.my*

Abstract: Corn starch (CS)-based films incorporated with nanocellulose fiber (CNF) and thymol (Thy) were prepared via the solution casting method. The combined effect of CNF and Thy on quality attributes of fresh chicken meat stored at 10 °C was investigated regarding visual appearance, weight loss, pH, and total viable count (TVC). In terms of visual appearance upon 7 days of storage, chicken meat samples exhibited shrinkage due to moisture loss, except for the sample wrapped in commercial film. However, the samples wrapped in CS/CNF/Thy films had better visual appearance, odor, and minimal pH change. Microbial populations were the lowest for sample wrapped in CS/CNF/Thy film compared to the CS and commercial films. In brief, the application of the active film considerably delayed the growth of microorganisms on chicken meat sample, increasing the food shelf life (<7 days) compared to control samples (<3 days) thus promising for active food packaging application.

Keywords—chicken meat; thymol; nanocellulose; starch film; quality attributes

INTRODUCTION

Fresh poultry meat, characterized by its high protein and moisture content, provides an ideal environment for microbial growth and oxidative processes, thereby being highly perishable[1]. These factors contribute to the loss of quality attributes and safety, resulting in domestic food waste, economic losses, and potential health hazards. To address these challenges, active packaging films, which incorporate active compounds such as antimicrobial agents, is a promising approach to preserving meat quality and prolonging shelf life[2]. For instance, gelatin/carrageenan-based nanocomposite films incorporated with tumeric essential oil able to reduce bacterial growth in chicken meat from 3.08 log CFU/g to 2.81 log CFU/g after 14 days of refrigerated storage[3]. Similarly, bacterial cellulose/polyvinyl alcohol films that contain Perilla essential oil emulsion displayed antimicrobial properties and mitigated the pH changes and oxidative rancidity of chilled beef, which resulted in extended shelf life[4].

Active packaging film can be designed using biopolymers such as corn starch (CS) as the base material, owing to its biodegradability, availability, and cost-effectiveness[5]. However, pure CS film often had poor mechanical and barrier properties. Thus, nanocellulose fiber (CNF) can be incorporated to overcome these limitations, while thymol (Thy) can provide additional functionalities. Thy contains a phenolic hydroxyl group which possesses significant antimicrobial and antioxidant properties[6]. The hydroxyl group of Thy and the hydrophobic character allow it to disrupt the microbial cell membrane, leading to leakage of proton flux and

intracellular constituents, and hence cell death[7]. Thy also bind with bacterial genomic DNA, disrupting protein synthesis that resulted in significant structural changes, ultimately leading to cell death[8].

Previous studies have utilized Thy in various packaging systems. In one study, Zabidi et al.[9] incorporated Thy in PLA/PBS-based films, and found that Thy at 9 wt.% loading able to show prolonged shelf life of chicken meat by having better odour and no fungal growth upon storage for 3 days at room temperature. Nonetheless, to our knowledge, there is limited research on the combined use of Thy and CNF in CS-based films for fresh poultry meat preservation. Only Othman et al.[10] have demonstrated the effectiveness of thymol in maintaining the quality of fresh beef wrapped in CS-based films, however, the study focused only on visual observation and microbial growth reduction. Thus, the present work aims to evaluate the effect of CS-based films incorporated with CNF and Thy in maintaining the quality attributes of fresh chicken meat in terms of visual appearance, weight loss, pH, and microbial growth reduction.

MATERIALS

Corn starch (33% amylose, 67% amylopectin), glycerol, thymol crystal (99%), Tween 20 (polyoxyethylene-20- sorbitan monooleate), methanol, and ethanol (99.7%) were obtained from R&M Chemicals Sdn. Bhd., Malaysia. The CNF suspension was purchased from Zoepnano Sdn. Bhd., Malaysia. Plate count agar and peptone water were obtained from Oxoid Limited, United Kingdom. The commercial plastic film and the chicken breast were purchased from a local supermarket, Hero Mart Sri Serdang, Malaysia.

EXPERIMENTAL

Preparation of films

The CS-based films (CS and CS/CNF/Thy) were prepared by solution casting method as described by Nordin et al.[11]. Briefly, CS was dispersed in distilled water (4% w/v) containing glycerol (25 wt.%) and gelatinized by heating at 90 °C for 30 min at using a hot plate stirrer (DAIHAN, Jakarta, Indonesia). Meanwhile, CNF/Thy emulsion was prepared by dispersing thymol (10 wt.% of CS) in 40 ml of distilled water containing CNF (1.5 wt.% of starch) at 50 °C. The concentration of thymol and CNF chosen in this study were based on prior studies by Othman et al.[10], where this formulation exhibited optimum overall performance including antibacterial activities. Tween 20 (fixed at 275 µL) was added as a surfactant and the emulsion was continuously stirred for 30 min at 1000 rpm. Then, the emulsion was added to the gelatinized film solution, dropwise at 50 °C with continuous stirring. Subsequently, the film solution underwent ultrasonication in ice bath (Qsonica, 500 W, 20 kHz, Newtown, CT) before casting (40 mL).

The CS film was prepared using the same procedure but without CNF and Thy. All film solution underwent drying in an air-conditioned room for 48 h and then placed in a ventilated oven (Memmert, USA) at 40 °C for 15 min. The dried films were peeled and conditioned in a desiccator containing saturated salt prior to further analysis.

Application of the films for fresh chicken meat preservation

The chicken meat was first sprayed with 75% ethanol to kill any available bacteria. Then the meat samples (3 cm x 3 cm) were enveloped in respective film (6 cm x 6 cm). In this part, a few treatments were made for comparison: samples wrapped with CS film, CS/CNF/Thy, and commercial film, individually. The samples were stored in a laboratory chiller at 10 °C for 7 days.

Evaluation of quality attributes of the chicken meat was conducted on days 0, 3, and 7, for the weight loss, pH, and total viable count (TVC). The visual appearance of the samples was photographed on each sampling day. The weight of the chicken meat samples was also determined using an analytical balance (Shimadzu, Japan). The percentage of weight loss was calculated according to equation (1).

$$\text{Weight loss (\%)} = \frac{\text{Weight}_i - \text{Weight}_t}{\text{Weight}_i} \times 100$$

where, weight_t represents weight of the sample on the sampling day and weight_i is the initial weight.

The pH changes of the chicken meat samples were determined by homogenizing 5 g of chicken meat sample in 45 mL of distilled water and macerated using probe homogenizer (DAIHAN, HG-15D, Korea) to produce a homogenized slurry[12]. Then, the pH of the homogenate was taken in triplicates by immersing a glass electrode of a pH meter (Mettler Toledo, China) in the slurry, and was averaged.

For the microbiological assessment, about 10 g of chicken meat sample was macerated in 90 mL of sterile 0.1% peptone water and then appropriate serial dilution of the homogenate was prepared. The TVC was determined using the pour plate method, using 0.1 mL of the diluent. After the molten media solidified, all plates were incubated at 37 °C for 24 h. Then, colony count was performed and expressed in log CFU/g.










Statistical analysis

Statistical analyses were conducted using Minitab software (Minitab Inc, State College, PA, USA). A p-value of <0.05 indicates a statistically significant difference between means.

RESULTS AND DISCUSSION

Based on Table 1, notable changes can be seen for samples in CS and CS/CNF/Thy films starting from day 3 onwards.

Table 1: Comparison of visual appearance of chicken meat samples over the storage time at 10 °C.

Films	Day 0	Day 3	Day 7
CS			
CS/ CNF/Thy			
Comm-ercial			

On day 3, the chicken meat sample packed in CS film exhibited notable shrinkage. The chicken meat sample in CS/CNF/Thy film exhibited similar behavior, but less pronounced than that of the CS film, indicating better film barrier against moisture loss with the presence of CNF. The presence of Thy could also have preserved the meat color better by minimizing oxidation and reducing the growth of microbes which responsible for the color changes. On day 7, the chicken samples in CS and CS/CNF/Thy films exhibited severe shrinkage and discoloration, however, CS/CNF/Thy film was slightly better at maintaining the visual appearance of the sample. Meanwhile, the chicken meat sample in commercial film remained the pinkish color and fresh look on days 3 and 7 but exhibited strong potent odor, which was linked to the presence of chicken 'juice' observed in the packaging[13]. These juices, when trapped within the commercial film packaging which has low permeability to water vapor, can provide a conducive environment for microbial growth[9,14] and the production of off-odors, contributing to the strong potent odor.

A general increasing trend in weight loss and pH was observed in all samples as the storage duration increased from day 0 to day 7 (see Fig. 1 and Fig. 2). The weight loss was mainly attributed to moisture loss whereas the pH changes were ascribed to the accumulation of alkaline substances such as amines and ammonia caused by the breakdown of proteins by the proteolytic enzymes of spoilage bacteria[15].

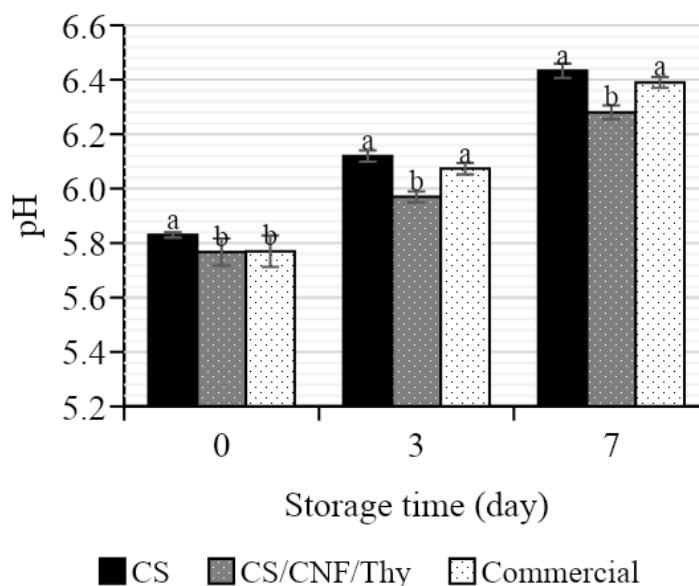


Fig 1: Comparison of pH values of chicken meat samples over the storage time at 10 °C.

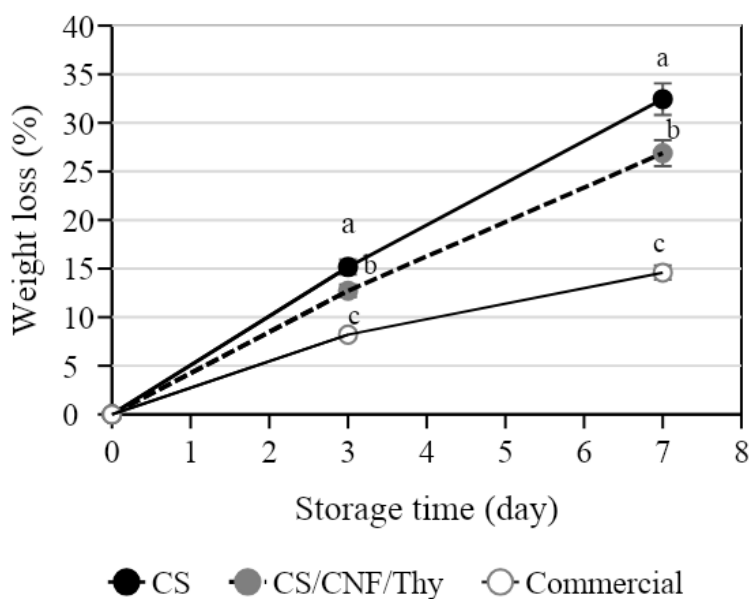


Fig 2: Comparison of percentage of weight loss of chicken meat samples over the storage time at 10 °C.

The samples in CS/CNF/Thy and commercial films had a slower increase in pH and weight loss ($p < 0.05$) compared to that of CS film which were attributed to the components present in the film formulation. In particular, the Thy possessed antibacterial activity, while the CNF and the highly branched structure of the commercial film could have induced additional barriers for moisture and gaseous exchange potentially influencing metabolic processes that impacted the pH and weight loss.

The TVC of the sample packed in CS/CNF/Thy film remained below the maximum permissible limit (7 log CFU/g)[16] up to day 3, and significantly lower than the samples in CS and LDPE films during the 7 days of storage (Fig. 3). This was attributed to the presence of Thy which possessed antibacterial activities that inhibited the growth of microbes, correlating to the results of pH. The efficiency of CS/CNF/Thy films in retarding the increase of microbial load in comparison to the CS and commercial films was also ascribed to the presence of CNF that acted as a physical barrier to provide controlled release of Thy[11]. This led to better retention of Thy, which has the potent phenolic hydroxyl group that created unfavourable conditions for microbial growth. Based on the TVC analysis, samples in CS and commercial films had a shelf life of <3 days, while it was <7 days for sample in CS/CNF/Thy film. These results proved the effectiveness of the release of Thy from the film upon contact with the chicken meat sample.

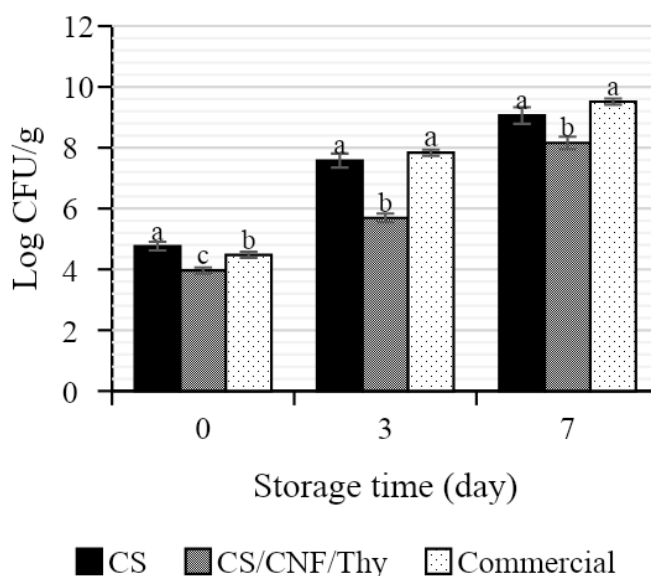


Fig 3: Comparison of TVC values of chicken meat samples over the storage time at 10 °C.

CONCLUSION

The findings proved the active functionality of CS-based film incorporated with CNF and Thy in maintaining the quality attributes of chicken meat stored at 10 °C. Such effects are influenced by the barrier properties and presence of active compounds in the film. Thus, CS/CNF/Thy film produced in this study is promising to be used as active packaging material. Future studies are necessary to explore the production of the active films in larger scale, ensuring the consistency in controlled release behaviour while maintaining cost-effectiveness.

ACKNOWLEDGEMENTS

This research was supported by the Fundamental Research Grant Scheme (FRGS), Ministry of Higher Education Malaysia (Vot no.: 5540711, Project no. FRGS/1/2024/TK05/UPM/02/9).

REFERENCES

- [1] A. Ahmadi, P. Ahmadi, A. Ehsani. Development of an active packaging system containing zinc oxide nanoparticles for the extension of chicken fillet shelf life. *Food Science & Nutrition*, 8(10), pp.5461-5473, 2020.
- [2] Y. Xu, N. Rehmani, L. Alsubaie, C. Kim, E. Sismour, A. Scales. Tapioca starch active nanocomposite films and their antimicrobial effectiveness on ready-to-eat chicken meat. *Food Packaging and Shelf Life*, 16, pp.86-91, 2018.
- [3] Ali, M. S., M. Haq, V. C. Roy, T. C. Ho, J. S. Park, J. M. Han, B. S. Chun. Development of fish gelatin/carrageenan/zein bio-nanocomposite active-films incorporated with turmeric essential oil and their application in chicken meat preservation. *Colloids and Surfaces B: Biointerfaces*, 226, 113320, 2023.
- [4] Y. Ma, Y. Cao, L. Zhang, Q. Yu. Preservation of chilled beef using active films based on bacterial cellulose and polyvinyl alcohol with the incorporation of Perilla essential oil Pickering emulsion. *International Journal of Biological Macromolecules*, 132118, 2024.
- [5] B. Wang, X. Xu, Y. Fang, S. Yan, B. Cui, A. M. Abd El-Aty. Effect of different ratios of glycerol and erythritol on properties of corn starch-based films. *Frontiers in Nutrition*, 9, p.882682, 2022.
- [6] M. J. Galotto, C. L. De Dicastillo, A. Torres, A. Guarda. Thymol: Use in antimicrobial packaging. In *Antimicrobial food packaging* (pp. 553-562). Academic Press. 2016.
- [7] E. Medina, N. Caro, L. Abugoch, A. Gamboa, M. Díaz-Dosque, C. Tapia. Chitosan thymol nanoparticles improve the antimicrobial effect and the water vapour barrier of chitosan-quinoa protein films. *Journal of Food Engineering*, 240, pp.191-198, 2019.
- [8] L. H. Wang, Z. H. Zhang, X. A. Zeng, D. M. Gong, M. S. Wang. Combination of microbiological, spectroscopic and molecular docking techniques to study the antibacterial mechanism of thymol against *Staphylococcus aureus*: membrane damage and genomic DNA binding. *Analytical and bioanalytical chemistry*, 409, pp.1615-1625, 2017.
- [9] N. N. Zainal, I. S. M. A. Tawakkal, M. S. M. Basri, S. H. Ariffin, M. N. Naim. Effect of thymol on properties of bionanocomposites from poly (lactic acid)/poly (butylene succinate)/nanofibrillated cellulose for food packaging application. *International Journal of Biological Macromolecules*, 251, 126212, 2023.
- [10] S. H. Othman, B. M. Wane, N. Nordin, N. Z. Noor Hasnan, R. A. Talib, J. N. W. Karyadi. Physical, mechanical, and water vapor barrier properties of starch/cellulose nanofiber/thymol bionanocomposite films. *Polymers*, 13(23), p.4060, 2021.
- [11] N. Nordin, S. H. Othman, R. Kadir Basha, S. Abdul Rashid. Controlled Release and Antibacterial Activity of Corn Starch-based Films Containing Nanocellulose and Thymol. *Physica Scripta*, 99, 075951, 2024.
- [12] S. M. Yimenu, J. Koo, B. S. Kim, J. H. Kim, J. Y. Kim. Freshness-based real-time shelf-life estimation of packaged chicken meat under dynamic storage conditions. *Poultry Science*, 98(12), pp.6921-6930, 2019.
- [13] N. Mohamad, M. M. Mazlan, I. S. M. A. Tawakkal, R. A. Talib, L. K. Kian, M. Jawaid. Characterization of active polybutylene succinate films filled essential oils for food packaging application. *Journal of Polymers and the Environment*, 30(2), pp.585-596, 2022.
- [14] C. Wang, A. Aji, Ethylene scavenging film based on low-density polyethylene incorporating pumice and potassium permanganate and its application to preserve avocados. *LWT*, 172, 114200, 2022.
- [15] Z. Chen, W. Tian, X. Qin, H. Wang, L. Tan, X. Liu. Chitosan/oxidized Konjac Glucomannan films incorporated with Zanthoxylum Bungeanum essential oil: A novel approach for extending the shelf life of meat. *International Journal of Biological Macromolecules*, 262, p.129683, 2024.
- [16] A. Ntzimani, A. Kalamaras, T. Tsironi, P. Taoukis. Shelf life extension of chicken cuts packed under modified atmospheres and edible antimicrobial coatings. *Applied Sciences*, 13(6), 4025, 2023.