ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF CHITOSAN FILMS MADE WITH TWO SOLVENTS AND GREEN TEA EXTRACT FOR POTENTIAL USE IN MEAT

A. Felician-Vega[1], A. Sánchez-Escalante[1], J. Lizardi-Mendoza[1], T. J. Madera-Santana[1], H. Santacruz-Ortega[2], M. A. de la Rosa-Alcaraz[1], D. Velásquez-Jiménez[1], and G. R. Torrescano-Urrutia[1*]

[1]Centro de Investigación en Alimentación y Desarrollo, A.C. Carretera a la Victoria Km 0.6, Hermosillo, Sonora, 83304 México.

[2]Universidad de Sonora, Departamento de Investigación en Polímeros y Materiales. Blvd. Luis Encinas y Rosales S/N. Hermosillo, Sonora, 83000 México; *Corresponding author email: gtorrescano@ciad.mx

I. INTRODUCTION

In recent years, the demand for natural alternatives as substitutes for synthetic additives and preservatives that are currently used in the food industry has grown considerably, including the search for oils and extracts of natural origin [1]. One added benefit of the inclusion of natural alternatives in the production of films and coatings, in contrast with conventional packaging, is that many natural alternatives contain active compounds with antimicrobial and antioxidant properties [2]. In particular, chitosan (Ch) and green tea (GT) have been found to have strong interactions with a polymer matrix, thereby increasing the mechanical properties and antioxidant capacity of films. The use of these substances may be a viable alternative for food preservation [3]. The objective of the present study was to determine the antioxidant and antimicrobial effects of Ch films incorporated with green tea extract (GTE) using acetic acid (AA) and lactic acid (LA) as solvents.

II. MATERIALS AND METHODS

Chitosan was dissolved in aqueous solutions of AA and LA (2% w/v; 25°C) at concentrations of 0.5% and 1% with agitation (400 rpm). Green tea extract was included at a concentration of 0.1%. Films were formed through casting. To measure the antioxidant capacity, 25 mg of each sample was dissolved in 3 ml of distilled water. The concentration of total phenols was determined using Folin-Ciocalteau reagent as an oxidizing agent [4]. The antiradical activity of the samples was based on the activity of stable radical 1,1'-diphenyl-2-picrylhydrazyl (DPPH) [5]. The growth kinetics of a Gram-negative microorganism (*Escherichia coli*) (EC) and a Gram-positive microorganism (*Staphylococcus aureus*) (SA) on prepared films were evaluated using the microdilution method and 96-well microplates [6].

III. RESULTS AND DISCUSSION

The incorporation of GTE increased ($P<0.05$) the phenolic content of the films (Figure 1A) compared to the control, and the utilized solvent and Ch concentration also affected phenolic content. Higher phenolic content was found in the treatments containing 1% Ch and LA as the solvent. Likewise, an increase ($P<0.05$) in the percentage of inhibition of DPPH radicals was observed (Figure 1B) in films incorporating GTE with respect to the control; this inhibition value was higher than those reported for synthetic antioxidants such as BHT (butyl hydroxytoluene). The type of solvent also affected ($P<0.05$) the inhibition values; higher values were observed in films elaborated with the LA solution. In particular, 0.5% Ch in LA solution with 0.1% GTE resulted in the highest value of oxidation inhibition. In addition, the microbial growth of the two strains tested on prepared films was reduced ($P<0.05$). A bacteriostatic effect was observed compared to the control, and greater inhibition of SA was presented. The incorporation of GTE also improved the inhibition of the evaluated microorganisms. The most significant effect was associated with solvent used. Films made with AA had a considerable inhibitory impact on EC, whereas films made with LA better inhibited SA. Therefore, the properties of residual solvents present in a polymeric matrix may affect the growth of the tested microorganisms. Notably, as pH reduces, the ion interactions with amine groups from Ch intensify, causing hydrolysis of peptidoglycans, which are constituents of the cell walls of microorganisms, although this mechanism should be further evaluated.

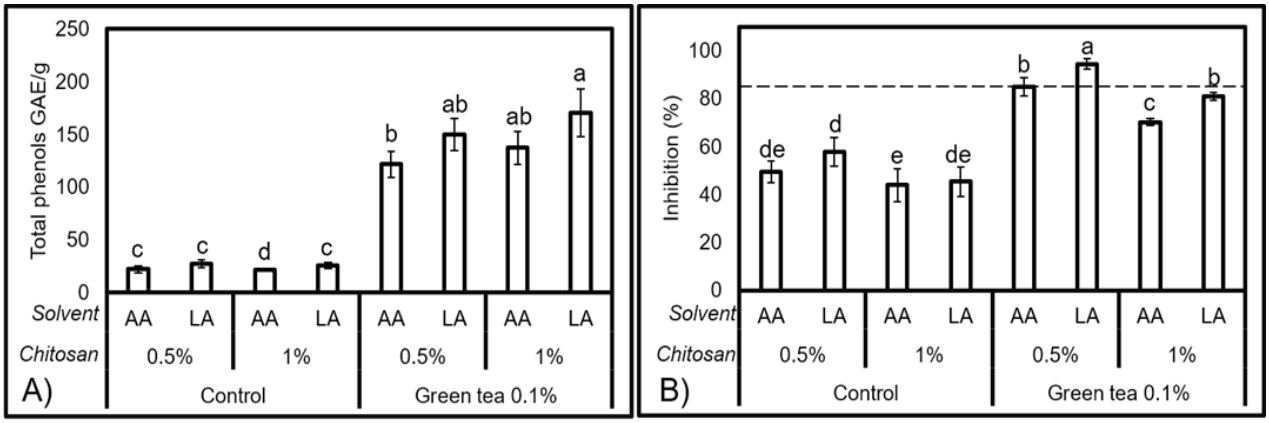

Figure 1. Antioxidant activity of chitosan films incorporating green tea extract: A) total phenolic content of chitosan films with green tea extract, B) DPPH scavenging of chitosan films with green tea extract. Values are given as the mean ± SD. Different letters indicate significant differences ($P \leq 0.05$). **(- - - -)** BHT (100 µg) [7].

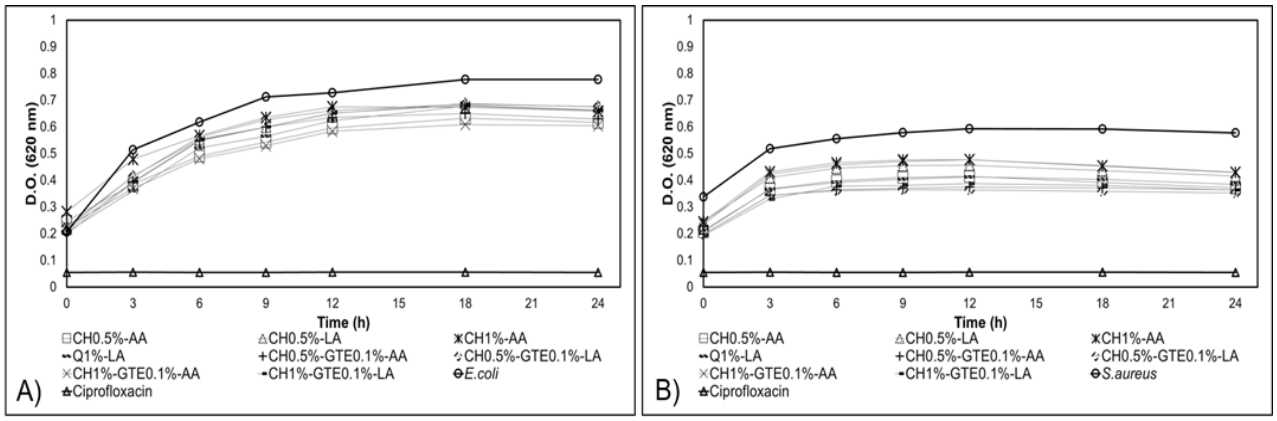

Figure 2. Antimicrobial activity of chitosan films incorporating green tea extract against A) *Escherichia coli* and B) *Staphylococcus aureus*. Values are given as the mean ± SD.

IV. CONCLUSION

The incorporation of GTE increased the antioxidant and antimicrobial capacity of elaborated films. The best results were observed for the treatments using LA as a solvent. Therefore, the use of GTE in combination with LA, represents a viable alternative for preserving and extending the shelf life of meat and meat products.

ACKNOWLEDGEMENTS

A. Felician-Vega acknowledges the fellowship received from CONACYT for his Master of Science thesis.

REFERENCES

1. Van Haute, S., Raes, K., Van der Meeren, P., & Sampers, I. (2016). The effect of cinnamon, oregano, and thyme essential oils in the marinade on the microbial shelf life of fish and meat products. Food Control 68: 30-39.
2. Siripatrawan, U., & Harte, B. R. (2010). Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. Food Hydrocolloids 24(8): 770-775.
3. Peng, Y., Wu, Y., & Li, Y. (2013). Development of tea extracts and chitosan composite films for active packaging materials. International Journal of Biological Macromolecules 59: 282-289.
4. Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture 16(3): 144-158.
5. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology 28(1): 25-30.
6. Wiegand, I., Hilpert, K., & Hancock, R. E. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature Protocols 3(2): 163.
7. Naveena, B. M., Sen, A. R., Vaithiyanathan, S., Babji, Y., & Kondaiah, N. (2008). Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. Meat Science 80(4), 1304-1308.