ANTIOXIDANT AND ANTIMICROBIAL EFFECT OF CHITOSAN-GREEN TEA FILMS IN PORK MEAT

Eduardo Montaño- Sánchez[1], Rey D. Vargas-Sánchez[1], Jaime Lizardi-Mendoza[2], Tomás J. Madera-Santana[3], Aarón F. González-Córdova[4], Armida Sánchez-Escalante[1], and Gastón R. Torrescano-Urrutia[1]

Centro de Investigación en Alimentación y Desarrollo, A.C. Carretera a La Victoria km 0.6, 83304, Hermosillo Sonora, México.
[1]Research Laboratory in Meat and Meat Products, [2]Biopolymers Laboratory, [3]Food Packaging and Shelf Life Laboratory, and [4]Chemistry and Biotechnology Dairy Laboratory

Abstract – Edible films were prepared by combining high molecular weight chitosan with aqueous green tea extract (GTE) at different concentrations (0, 0.1 and 0.5%). The effect of GTE concentrations and film-forming solution was determined by measuring mechanical properties, total phenolic content (TPC), total flavonoid content (TFC) and radical scavenging assay (DPPH). Films were also applied to the surface of pork meat (M. *L. dorsi*) 24 h *postmortem*, which was stored at 0 °C under darkness for 25 days. The results suggested that incorporation of GTE into chitosan films improved mechanical barrier properties, enhancing the polyphenolic content and antioxidant and antimicrobial activity of the films. The incorporation of green tea in the chitosan matrix led to a reduction in lipid oxidation (Lox), color changes and microbial counts in comparison to the control samples (P<0.05).

Key Words – Green tea extract, Chitosan film, antioxidant and antimicrobial activity.

I. INTRODUCTION

Lipid oxidation and foodborne pathogens are the principal cause of quality loss in pork meat. Packaging systems can help reduce risks to food safety caused by the oxidation by-products of lipids and foodborne pathogens by controlling post-processing contamination on food surfaces [1]. Chitosan, a linear polysaccharide, is a diacetylated derivative of chitin. In addition to its antioxidant and antimicrobial properties, dilute acid solutions of chitosan have good film-forming properties. Chitosan films present a very high hygroscopicity and thus may lose their physical integrity in the presence of moisture. Due to this restriction, films made from chitosan cannot completely replace synthetic polymer films in food packaging applications. However, chitosan layer can be used as an efficient carrier for other bioactive compounds, such as antimicrobial substances [2,3]. Green tea (*Camellia sinensis* L.) is a source of polyphenolic compounds that have strong antioxidant and antimicrobial activity. Catechins are the major polyphenols in tea leaves in addition to the presence of flavonols and flavones [4]. A number of recent studies have dealt with extending the functional properties of biodegradable films by adding different compounds with antioxidant or antimicrobial activity in order to yield a biodegradable, active packaging material [5]. Therefore, in this study, the antioxidant and antimicrobial effectiveness of chitosan-green tea films adhered to pork meat to extend its shelf life was evaluated.

II. MATERIALS AND METHODS

Bioactive compounds of green tea were extracted with water (80 °C), concentrated under reduced pressure and lyophilized. The chitosan solution was prepared by dissolving chitosan in an aqueous solution (1%, v/v) of acetic acid to reach a final concentration of 1% (w/v) [2]. Green tea extract (GTE) was added to chitosan solution at a concentration of 0.0, 0.1, and 0.5% (v/v) in order to evaluate *in vitro* antioxidants (total phenolic content (TPC), total flavonoid content (TFC), radical scavenging assay (DPPH) and antimicrobial effectiveness (minimum inhibitory concentration, MIC). Color and mechanical properties (EM, elastic modulus; N, elastic limit; Elong., elongation) were measured in each film solution [2]. Pork meat (M. *L. dorsi*) 24 h *postmortem* was obtained from a local processor and the surface of meat was coated with the films and stored at 0 °C under darkness for 25 days for subsequent quality analysis. Data were

tested using the NCSS07 statistical package through ANOVA analysis followed by a Tukey post-hoc test ($P<0.05$).

III. RESULTS AND DISCUSSION

Phenolic compounds are one of the major groups found in plants and have been reported to contain biological properties that function as antioxidant and antimicrobial agents [2,4]. GTE was incorporated to chitosan solution and tested *in vitro* to determine its antioxidant (AOX) and antimicrobial (AMI) activity. Results showed that GTE presented high TPC (equivalent to 582.7 mg gallic acid/g extract), TFC (152.1 mg rutin /g extract) and a DPPH value of 87.8% at 100 µg/ml. The GTE incorporated in the chitosan solution (GTE+chi) slightly enhanced AOX although mainly DPPH: GTE+chi 0.5>0.1 >0.05%. For the AOX of the 0.5% GTE concentration (100 µg/ml), TPC, TFC and DPPH values were 3.3 mg of gallic acid equivalent, 0.75 mg of rutin equivalent and 92.5%, respectively.

Results also indicate that GTE (0.5%) presented high antimicrobial activity against *Staphylococcus aureus* ATCC29213B (99%), *Listeria innocua* ATCC33090 (94%), *Escherichia coli* ATCC25922 (45%) and *Salmonella choleraesuis* ATCC10708 (40%); while GTE+chi GTE (0.5%) showed significant inhibition of *S. aureus* (90%), *L. innocua* (90%), *E.coli* (98%) and *S. choleraesuis* (97%). These results indicate that GTE is rich in polyphenolic compounds; previous research on AOX and AMI of GTE suggest that the associated biological properties could be due to presence of polyphenolic compounds [2].

The GTE concentration affected ($P<0.05$) the color of the film surface (Table 1). Lightness of the film (L* values) decreased from 34.67 to 31.62 %, but a* (redness) and b* (yellowness) increased from -0.21 to 2.86 and -0.25 to 6.04, respectively. The mechanical properties (resistance and extensibility) of chitosan films when incorporated with different GTE concentrations were significantly affected when compared to the control film ($P<0.05$). For EM, addition of GTE increased its parameters. This result possibly suggests that aqueous-GTE did not act as a plasticizing agent. However, N value increased at high GTE concentrations, increasing the mobility of the polymer matrix, which in turn increased film elongation. The improvement of the mechanical properties of the films incorporated with GTE may be therefore attributed to the interaction of the chitosan matrix with the polyphenolic compounds of GTE [2].

Additionally, the efficacy of GTE as an ingredient to inhibit Lox, color degradation and microbial growth in pork chops was assessed (Table 2). Over the course of the study, pH values increased gradually with increasing storage time ($P<0.05$). However, all treatments showed pH values characteristic of fresh meat 5.5-5.7 [6]. Lox levels, measured by reaction with TBA (thiobarbituric acid), were higher for the control and chitosan treatments ($P<0.05$) over the course of 25 days, while TBARS was significantly reduced in samples treated with 0.1 and 0.5% GTE. However, all treatments demonstrated TBARS values less 1 mg MDA/kg sample, which indicated that pork meat did not exhibit rancid flavor [7]. Meat color influences acceptability and plays a large role in the decision of the purchaser [7,8], and it was found that GTE addition in chitosan films did not affect the L* and b* values ($P>0.05$). On day 25, the average L* and b* values were 53.5 and 12.5, respectively, while on the same day, 0.1 and 0.5% GTE treatments showed a slight decrease of a* (2.24 and 2.28, respectively), indicating a lower loss of red color.

Mesophilic and psychrotrophic bacteria increased over the course of storage time ($P<0.05$). On day 25, pork meat treated with chitosan and 0.1 and 0.5% GTE films had the lowest mean flora counts during the storage period at 3.15 and 3.64 CFU/g for mesophilic and psychrotrophic bacteria, respectively. The bacterial counts did not exceed sanitary specifications [9], defined as the maximum permissible amount (6 $\log_{10}$ CFU/g). Antibacterial action of polyphenolic compounds present in GTE could be due to their ability to (1) inhibit cell division, (2) destroy cell cytoplasm and membranes (bacteriolysis), causing the rupture of cell components and changes in the fatty acids of membranes and phospholipid content, and (3) inhibit DNA and RNA synthesis [10]. These results confirmed that pork meat treated with chitosan films containing GTE, rich in phenolic compounds, reduced Lox, color changes and microbial growth in

fresh meat over the course 25 days when compared with the control samples.

Table 1 Color and mechanical properties of films.

Parameter	Film sample			
	Chi	0.05%	0.1%	0.5%
L*	34.67^{c}	34.36^{b}	34.32^{b}	31.62^{a}
a*	-0.21^{a}	0.53^{b}	1.04^{c}	2.86^{d}
b*	-0.25^{a}	2.99^{b}	4.67^{c}	6.04^{d}
EM	1559^{a}	3434^{b}	3778^{c}	4325^{d}
N	57.51^{a}	75.62^{b}	87.85^{c}	84.13^{c}
Elong.	10.93^{c}	8.59^{b}	11.02^{c}	5.16^{a}

Chi, chitosan; EM, elastic modulus; N, elastic limit; Elong., elongation. Different superscripts (a-d) in each sample differ significantly ($P<0.05$).

Table 2 Lox, color and levels of microbial growth of pork meat during storage time.

Analysis	d	Control	Chi	0.1%	0.5%
pH	0	5.62bA	5.44aA	5.40aA	5.13aA
	10	5.63bA	5.50aA	5.47aA	5.54aA
	20	5.70bA	5.53aB	5.60aB	5.56aB
	25	5.77cB	5.53aB	5.63bB	5.57bB
TBARS	0	0.05^{aA}	0.05^{aA}	0.05^{aA}	0.04^{aA}
	10	0.05^{bA}	0.06^{bA}	0.04^{aA}	0.04^{aA}
	20	0.02^{bB}	0.09^{bB}	0.05^{aA}	0.04^{aA}
	25	0.09^{bB}	0.13^{bB}	0.05^{aA}	0.04^{aA}
a*	0	1.78^{aA}	1.28^{aA}	1.72^{aA}	2.02^{aA}
	10	1.93^{aA}	1.58^{aA}	2.13^{aA}	2.39^{aA}
	20	2.24^{aA}	2.15^{aA}	2.40^{aA}	3.05^{aA}
	25	2.46^{aA}	2.36^{aA}	2.24^{aA}	3.18^{bA}
Mesophi.	0	1.09^{aA}	1.12^{aA}	1.37^{aA}	1.43^{aA}
	10	3.21^{bB}	2.62^{aB}	2.57^{aB}	2.37^{aB}
	20	5.03^{bC}	2.88^{aC}	3.04^{aC}	2.88^{aC}
	25	6.32^{bD}	3.07^{aC}	3.25^{aC}	3.15^{aC}
Psychro.	0	1.72^{aA}	1.12^{aA}	1.12^{aA}	1.00^{aA}
	10	3.29^{cB}	2.55^{bB}	2.52^{bB}	2.10^{aB}
	20	5.44^{bC}	3.13^{aC}	2.99^{aC}	3.40^{aC}
	25	6.12^{bD}	3.72^{aC}	3.64^{aC}	3.56^{aC}

d, day of sampling; Chi, chitosan; Mesophi, mesophilic; Psychro, psychrotrophic. Different superscripts (a-d) within the same sampling day and (A-B) through storage time differ significantly ($P<0.05$).

CONCLUSION

In this study, the current findings demonstrated that the application of chitosan film with GTE as an antioxidant and antimicrobial agent in pork meat stored at 0 ℃ without illumination can be effectively used to reduce Lox and color changes, as well as microbial growth that occurs during chilled storage. This work has demonstrated the great potential of chitosan films with added GTE as a preservative to increase the shelf life of fresh meat.

ACKNOWLEDGEMENTS

The authors thank the technical assistance of Livier Zavala-Cárdenas and Brisa del Mar Torres-Martínez.

REFERENCES

1. Vargas, M., Albors, A., & Chiralt, A. (2011). Application of chitosan-sunflower oil edible films to pork meat hamburgers. Procedia Food Science: 39-43.
2. Torlak, E., & Sert, D. (2013). Antibacterial effectiveness of chitosan–propolis coated polypropylene films against foodborne pathogens. International Journal of Biological Macromolecules 60: 52-55.
3. 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: 770-775.
4. Graham, H. N. (1992). Green tea composition, consumption, and polyphenol chemistry. Preventive medicine 21: 334-350.
5. Suppakul, P., Miltz, J., Sonneveld, K., & Bigger, S.W. (2003). Active packaging technologies with an emphasis on antimicrobial packaging and its applications. Journal of Food Science 68: 408-420.
6. Torrescano G, Sánchez-Escalante A, Jiménez B, Roncalés P, Beltrán JA. 2003. Shear values of raw samples of 14 bovine muscles and their relation to muscle collagen characteristics. Meat Science 64: 85-91.
7. Ockerman, H.W. (1985). Quality control of post-mortem muscle and tissue. Department of Animal Science, Ohio State University. Columbus, OH USA. Vol. 1. p91.
8. Girolami, A., Napolitano, F., Faraone, D., & Braghieri, A. (2013). Measurement of meat color using a computer vision system. Meat Science 93: 111-118.

9. NOM-110-SSA1. 1994. Norma Oficial Mexicana. Bienes y servicios. Preparación y dilución de muestras de alimentos para su análisis microbiológico.
10. Shan, B., Cai, Y.Z., Brooks, J.D., Corke, H. 2007. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. International Journal of Food Microbiology 117:112-119.