PE4.12 Controlled release of catechin from edible zein films intended for meat bioactive packaging 37.00

Ahmet Yemenicioðlu (1) ahmetyemenicioglu@iyte.edu.tr, Ý Arcan(1), ,
(1)Izmir Institute of Technology, Turkey

***Abstract*—Catechins are potent natural antimicrobial and antioxidants which can be utilized in place of chemical additives to increase quality and shelf-life of meat and meat products. In this study, controlled release of (+) catechin, the basic one of catechins, was studied from zein films intended for meat bioactive packaging. The release rate of catechin from zein films can be reduced by incorporation of carnauba wax and soy lechitin into films by the homogenization method. The reduced diffusion rates were achieved; (1) by increased hydrophobicity and tortuosity of films by incorporated wax particles and (2) network formation within the film matrix by H-bonding of catechin to zein and wax surfaces. The increase of catechin concentration enhanced the network and the barrier effect against diffusion of catechin. The formed network gave a considerable flexibility and elasticity to zein films which brittleness is a major problem in their use in meat products as self-standing films. The films showed soluble and immobilized antioxidant activity as well as antimicrobial activity on test microorganism *Listeria innocua*. This study forms the basis to use catechins for meat bioactive packaging.**

Authors are with Izmir Institute of Technology, Department of Food Engineering, Gulbahce Koyu, 35430 Urla, Izmir. (corresponding author to provide phone: +90-232-7506292; Fax: +90-232-7506196; e-mail: ahmetyemenicioglu@iyte.edu.tr)

***Index Terms*— antimicrobial, antioxidant, controlled release, phenolic compound, packaging, edible film**

I. INTRODUCTION

…In meat industry, antimicrobials or antioxidants are mostly mixed into initial formulations. Dipping is also a method to apply preservatives. However, these traditional applications have some limitations such as the cease of protection effect once the active compounds are consumed in complex reactions in food and lack of selectivity to target the food surface where most microbial and oxidative spoilage occur intensively. The use of active packaging systems with controlled release properties is an alternative method to overcame these limitations since this provides continuous release of additives from packaging materials to food surface, thus, maintaining their critical concentration at the surface necessary for inhibiting the microbial growth or oxidative change [1].

Controlled release systems have been developed and used extensively for pharmaceutical applications. However, the studies in the field of food packaging are very limited. Different chemical antimicrobials and antioxidants can be incorporated into plastic, biodegradable and/or edible packaging materials [1, 2]. However, due to the health concerns of the consumers and environmental problems, producers are now particularly interested in the use of bioactive compounds in edible and biodegradable packaging materials. The catechin and its derivatives are potent natural antimicrobial and antioxidants that can be utilized in place of chemical antioxidants and antimicrobials to increase quality and shelf-life of meats, poultry, fish and their products [3, 4, 5, 6, 7, 8, 9]. In fact, recently, catechin has been successfully used as antimicrobial and antioxidant for bioactive packaging of a meat product [9]. The use of natural phenolic compounds in foods is particularly encouraged since they had many different benefits on human health including protective effects against cancer, cardiovascular disease, diabetes and degenerative diseases [10]. In fact, the supplementation of feeds with tea catechins is also employed to improve animal health and obtained meat quality in respect to oxidative stability during storage [4, 11].

In this study, bioactive zein films have been developed by incorporation of (+)catechin, a basic catechin, into zein films. The controlled release of catechin from zein films was achieved by dispersing hydrophobic carnauba wax particles into zein films by high speed homogenization. This strategy applied previously by Ozdemir and Floros [12] for controlled release of potassium sorbate targets increasing of film hydrophobicity to slow down its water intake (swelling) and to create a tortuosity against diffusion of antimicrobial agent. However, different from the previous study we conducted the homogenization process in presence of surface active compound soy lechitin. This aims maintaining reduced size of wax particles during film making and creating polar sites at wax and zein surfaces for film cross-linking due to H-

bonding of catechin. The H-bonding of catechin can also occur with protein peptide carbonyl groups and the network formed may improve the controlled release properties of film. It was also reported that the network formation in protein based films by phenolic compounds improves the film mechanical properties [13]. The brittleness and lack of elasticity are main problems in use of zein as a self standing film. This study forms the basis to develop phenolics incorporated zein based self-standing films, coatings or casings with improved controlled release properties for bioactive packaging of meat, poultry and fish and their products.

II. MATERIALS AND METHODS

Materials.

Zein and (+) catechin were from Sigma Chem Co. (St. Louise, Mo, USA). Carnauba wax (No.1, refined) was form Sigma-Aldrich (St. Louise, Mo, USA), and soy lechitin was from Merck (Darmsdadt, Germany). All other chemicals were reagent grade.

Film making.

The zein films were prepared as described by Padgett et al. [14]. Briefly, 1.4 g zein was dissolved with 8.1 mL of ethanol (97 %) by mixing slowly with a magnetic stirrer for 25 min. 0.4 mL glycerol was added and stirring was continued for 5 min. Then, carnauba wax was added (5% (w/w) of zein) and the temperature was increased until boiling initiated. The film solution was boiled for 5 min without any stirring, cooled for 10 min at room temperature and lecithin was added (5% (w/w) of zein). The mixture was further stirred 2.5 min to dissolve lechitin and different amounts of catechin was added. This mixture was then homogenized (Heidolph, Germany, Type 8 F rotor, 6.6 mm tip) at 10000 rpm for 4 min and cast into a glass template (W x L x H: 8.5 x 8.5 x 0.4 cm). The films were dried at 25^{o}C for 12h, peeled and 4 x 4 cm pieces cut from their middle were used in tests. The thicknesses of films (control: 131.8±2.0 µm, wax and lechitin containing films: 119.2±1.9 µm, catechin containing films: 127.8±5.4 µm, wax, lechitin, catechin containing films: 128.02±12.9 µm) were determined by SEM (Philips XL 30S FEG, FEI Company, Netherlands).

Total flavonoid content.

The catechin concentration was determined by the colorimetric method given by Meyers et al [15]. An 250 µL of sample was mixed with 1 mL deionized water and 75µL of $NaNO_2$ (5%). The mixture was mixed and incubated. At the 5th min of incubation, 75µL of $AlCl_3$ (10%) was added, and at the 6th min of incubation 0.5 mL of 1M NaOH and 0.6 mL of deionized water was added. The formed color was then measured by a spectrophotometer at 510 nm. The calibration curve was prepared by (+) catechin. Average of three measurements was used in calculations.

Release tests.

The release tests were conducted with 4x4 cm film pieces in glass Petry dishes (10 cm diameter) containing 50 mL of deionized water and kept in an orbital shaker working at 80 rpm and 4^{o}C. The tests were continued until reaching the equilibrium. For sampling, 750 µL water was taken at different time periods and flavonoid content was assayed for three times. Evaporation from plates was minimized by covering plates with their lids, flexible plastic films and aluminum sheets.

S*oluble antioxidant capacity of films.*

Soluble antioxidant capacity of films was based on their total released catechin content during the release tests. The antioxidant activity of pure (+) catechin was determined by the area under the curve (AUC) value (16.1 micro mol trolox/mg) according to the standard ABTS method of Re et al [16]. This method is based on monitoring the percent inhibition of blue colored ABTS free radical from its decolorization by the antioxidant with a spectrophotometer at 734nm (test periods: 1,3,6,9,12,15 min). The results were expressed as micro mol trolox equivalents of catechin released per cm^2 of films.

Immobilized antioxidant capacity of films.

For this purpose films reached to equilibrium in release tests were washed two times with 100ml of deionized water (2x50ml) for 60 min by shaking at 80 rpm to remove residual soluble catechin at their surface. Two 2x2 cm pieces were cut from the films and placed into separate Petri dishes containing 50 mL ABTS free radical solution. The reaction was conducted at 30^{o}C by shaking at 80 rpm and the percent inhibition of ABTS solution in 15 min was determined at 734 nm. The antioxidant capacity was determined as trolox equivalent as micro mol trolox/cm^2. Average of two measurements was used in calculations.

Antimicrobial activity of films.

The overnight culture of *L. innocua* (NRRL B-33314) was prepared in nutrient broth at 37 ^{o}C. Discs (9 to 15) (1.3 cm in diameter) were prepared from films by a cork borer under aseptic conditions. During tests, 3 discs were placed carefully onto each Petri dish containing nutrient agar on which 0.1 ml culture was spread. The average number of cells used in tests was 63 x 10^7 cfu/ml. Petri dishes were incubated at 37 ^{o}C for 48 h and results were evaluated by carefully removing the discs from agar surfaces and comparing clarity of disc contact locations with those of control films.

Mechanical properties of the films.

Tensile strength, elongation at break, and elastic modulus were determined using a Texture Analyser TA-XT2 (Stable Microsystems, Godalming, UK) according to ASTM Standard Method D 882-02 [17]. The films were used in tests directly following drying. Films were cut into 5 mm wide and 80 mm length strips, initial grip distance was 50 mm, crosshead speed was 50 mm/min. At least seven replicates of each film were tested.

III. RESULTS AND DISCUSSION

Controlled release mechanism.

The characteristic film structure of zein consists of a meshwork composed of doughnut structures formed by asymmetric rods joined to each other [18]. In this work, to obtain controlled release properties, standard zein films were incorporated with carnauba wax by means of high speed homogenization. The controlled release in this method was achieved by slowing down of film water intake (swelling) due to its increased hydrophobicity. Moreover, formation of highly hydrophobic wax particles within film matrix increased film tortuosity against diffusion of antimicrobial compound [12]. Different from the previous studies we conducted the homogenization process in presence of surface active compound lechitin. This aimed formation of negatively charged carboxyl groups at surfaces of wax globules and prevention of increase in wax globule size due to charge-charge repulsion. It was also expected that the hydrophobic tails of lechitin also bind and buried into hydrophobic zein rods, and this increased the number of carboxyl groups in the film matrix. It is well known that the phenolic hydroxyl groups are capable to form H-bonding with peptide carbonyl groups of proteins and carboxyl groups such as those formed by lechitin [19]. Therefore, the catechin which contain five hydroxyl groups can form extensive H-bonding among asymmetric zein rods and wax globules to create a network (possible interactions: zein-zein, wax-zein, wax-wax) and contribute to controlled release properties of films.

Effect of wax and lechitin on release profiles.

The release profiles of different zein films containing 1.5 mg catechin/cm^2 were given in Fig. 1. It is clear that the catechin release rates from control zein films and films containing lechitin are similar and greater than those of other films. This result clearly showed the low diffusion-barrier effect of potential network formed by H-bonding among asymmetric zein rods. It seems that the catechin concentration of these films is not sufficient to form extensive H-bonding to create a diffusion-barrier effect. The incorporation of wax into films reduced the release rates of catechin from zein films considerably. However, it appears that some catechin entrapped within wax aggregates in these films and little catechin diffusion occurred at the later stages of diffusion. On the other hand, the initial catechin diffusion was slowest when wax and lechitin were incorporated into zein films in combination. It is also worth to note that this film showed the greatest amount of catechin release at the later stages of release test. It seems that the lechitin prevented aggregation of wax particles and trapping of catechin.

Effect of catechin concentration on release profiles.

The catechin concentration of the films was increased to obtain more intensive H-bonding and improve controlled release properties (Fig. 2). In films containing 0.75 mg catechin/cm^2, the release rate of catechin in control film is quite similar with that of zein film incorporated with wax and lechitin. However, as the catechin concentration increased, the rate of catechin release from wax and lechitin containing films got lower than those of the respective controls. For example, in films containing wax, lechitin and 3 mg catechin/cm^2, the amount of catechin released in 3 days was almost 47% less than that released from corresponding standard zein films.

Antioxidant potential of films.

The soluble and immobilized antioxidant activities provided by different films were given in Table 1. In general, films containing wax and lechitin yielded higher soluble antioxidant activities than films lacking these components. It seems that the use of surface active agent lechitin reduced the trapping of catechin within hydrophobic zein film matrix and among wax globule aggregates. The films containing same amounts of phenolic compound also showed similar immobilized antioxidant activities, except at 2.25 mg catechin/cm^2 which standard zein films showed moderately higher antioxidant activity. These, results showed the reduced release rates of catechin in wax and lechitin containing films without any trapping.

Antimicrobial activity of films.

The zein films lacking wax and lechitin but containing catechin did not form measurable zones around discs tested, but lack of microbial developpment at disc locations compared to control disc locaitons clearly indicated antimicrobial activity of these films at contact surfaces (Fig. 3A and B). All wax and lechitin containing zien discs expanded and melted at the icubation temperature. Therefore, this increased the disc area and masked the formed zones. When discs of wax and lechitin containing films were removed from the agar surfaces, some clear zones were identified around the residues melted and left at initial disc locations (Fig. 3C and D). These results clearly showed the presence of some antilisterial activity at film contact surfaces.

Mechanical properties of films.

As seen in Table 2, the use of catechin reduced the tensile strenght of films, but it turned the highly brittle zein films to elastic ones. This clearly showed the increased networking in the films by use of catechin. The greatest elongation was observed in films lacking wax and lechitin. Thus, it seems that the wax globules in the films reduced the elongation of films by breaking the networking.

IV. CONCLUSION

The increase of microbial and oxidative quality of meat and meat products by natural phenolic compounds including catechins have been shown with numerous studies. This work showed the controlled release of (+)catechin, the basic catechin, from zein films intended for meat bioactive packaging. By use of catechin, edible film gained antimicrobial and antioxidant activity. Moreover, the network formed within film matrix by phenolic compound eliminated the classical brittleness problem of zein films. The self-standing films, coatings or casings of zein containing phenolic compounds have a great potential for bioactive packaging of meat and meat products.

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REFERENCES

[1] Appendini, P., & Hotchkiss, J.H. (1997). Immobilization of lysozyme on food contact polymers as potential antimicrobial films. Packaging Technology and Science, 10, 271-279.

[2] 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 (2), 408-420.

[3] O'Grady, M.N., Maher, M., Troy, D.J., Moloney, A.P., Kerry, J.P. (2006). An assessment of dietary supplementation with tea catechins and rosemary extract on the quality of fresh beef. Meat Science, 73, 132-143.

[4] Yilmaz, Y. (2006). Novel uses of catechins in foods, Trends in Food Science and Technology, 17, 64-71.

[5] Saucier, C.T., Waterhouse, A.L. (1999). Synergetic activity of catechin and other antioxidants. J.ournal of Agricultural and Food Chemistry, 47 (11), 4491-4494.

[6] Almajao, M.P., Carbo, R., Jimenez, J.A.L., Gordon, M.H. (2008). Antioxidant and antimicrobial activities of tea infusion. Food Chemistry, 108, 55-63.

[7] Vaquero, M.J.R., Alberto, M.R., Nadra, M.C.M. (2007). Influence of phenolic compounds from wies on the growth of *Listeria monocytogenes*. Food Control, 18, 587-593.

[8] Vaquero, M.J.R., Alberto, M.R., Nadra, M.C.M. (2007). Antibacterial effect of phenolic compounds from different wines. Food Control,18, 93-101.

[9] Ku, K.J., Hong, Y.H., Song, K.B. (2008). Mechanical properties of *Gelidium corneum* edible film containing catechin and its applications in sausages. Journal of Food Science, 73 (3), 217-221.

[10] Crespy, V., Williamson, G. (2004). A review of the health effects of green tea catechins in in vivo animal models. The Journal of Nutrition, 18, 3431-3440.

[11] Tang, S.Z., Kerry, J.P., Sheehan, D., Buckley, D.J., Morrissey, P.A., (2001). Antioxidative effect of dietary tea catechins on lipid oxidation of long-term frozen stored chicken meat. Meat Science, 57, 331-336.

[12] Ozdemir, M., Floros, J.D. (2003). Film composition effects on diffusion of potassium sorbate through whey protein films. Journal of Food Science, 68, 511-516.

[13] Qu, S., Weng, Y., Tang, S., Huang, C., Jackson, M.G. Role of ferulic acid in preparing edible films from soy protein isolate. Journal of Food Engineering, 70, 205-210.

[14] Padgett, T., Han, I.Y., & Dawson, P.L. (1998). Incorporation of food-grade antimicrobial compounds into biodegradable packaging films. *Journal of Food Protection*, 61 (10), 1330-1335.

[15] Meyers, K.J., Wtkins, C.B., Pritts, M,P,, Liu, R.H. (2003). Antioxidant and antiproliferative activities of strawberries. Journal of Agricultural ans Food Chemistry. 51, 6892-6892.

[16] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26, 1231–1237.

[17] ASTM, 2002. Standard test method for tensile properties of thin plastic sheeting- D882-02. In: Annual Book of American Standard Testing Methods. ASTM, Philadelphia, PA.

[18] Gou, T., Liu, Z., An, H., Li, M., & Hu, J. (2005) Nano-structure and properties of maize zein studied by atomic force microscopy. Journal of Cereal Science, 41, 277-281.

[19] Damodaran, S. (1996). Amino acids, peptides and proteins. In O.R. Fennema, FoodChemistry (pp. 347). New York: Marcel Decker Inc.

Table 1. Effect of film composition on antioxidant potential

Film components[a]			Antioxidant potential (micro mol trolox/cm^2)	
			Soluble	Immobilized
C	W	L		
1.5	-	-	12.4	0.27
1.5	5	5	14.1	0.26
2.25	-	-	20.2	0.34
2.25	5	5	21.9	0.26
3.0	-	-	27.4	0.31
3.0	5	5	29.7	0.33

[a]C: catechin (mg/cm^2); W: wax and L:lechitin (% of zein)

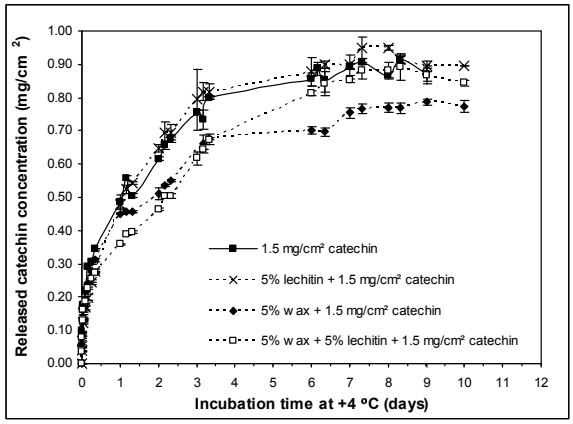

Fig .1 Effect of different components on release profiles

Table 2. Mechanical properties of different films

Film components[a]			Tensile strenght (MPa)	Elongation (%)	Elastic modulus (MPa)
C	W	L			
-	-	-	0.0160 ±0.0022	1.8 ±0.3	0.0090 ±0.0012
-	5	5	0.0139 ±0.0023	1.2 ±0.2	0.0103 ±0.0006
3	-	-	0.0063 ±0.0008	132 ±30	0.0021 ±0.0002
3	5	5	0.0044 ±0.0005	64 ±6.0	0.0021 ±0.0014

[a]C: catechin (mg/cm^2); W: wax and L:lechitin (% of zein)

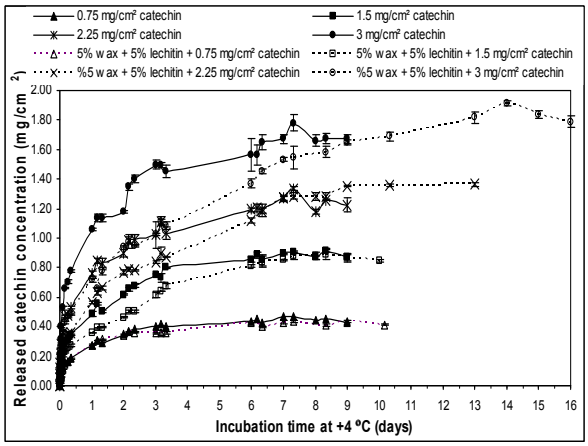

Fig. 2. Effect of catechin content on release profiles

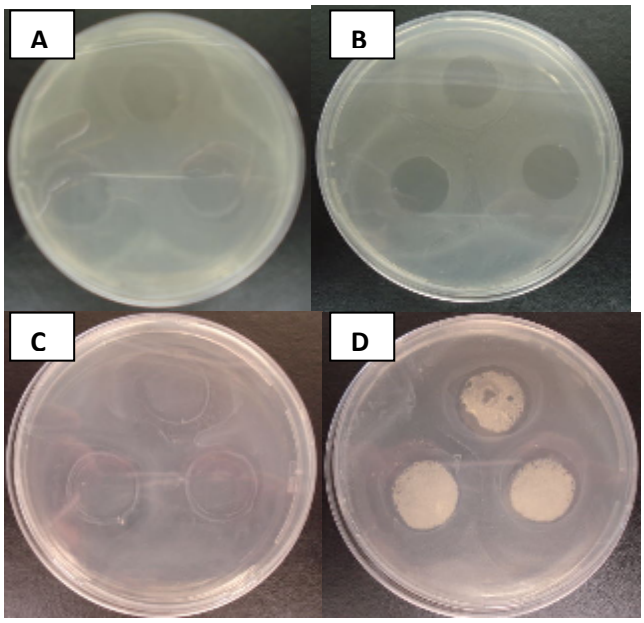

Fig. 3. Antimicrobial activiy of zein films on *L.innocua*.
A: control, B: 3 mg/cm^2 catechin, C: control wax+lechitin, D: wax+lechitin+3 mg/cm^2 catechin.