INFLUENCE OF NOVEL ACTIVE NANOCOMPOSITE POLYETHYLENE FILMS INCORPORATED WITH NANOCLAYS AND PLANT EXTRACTS IN CONTROLLING LIPID OXIDATION AND GROWTH OF *ESCHERICHIA COLI* O157:H7 IN GROUND BEEF

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Abstract – In this study, novel active nanocomposite polyethylene films incorporated with montmorillonite (MMT) or halloysite (H) nanoclays and natural plant extracts (thymol (THY), eugenol (EUG) and carvacrol (CRV)) were produced by melt mixing and blow molding method. Effect of those films as packaging materials for ground beef stored at 5 ºC in the control of lipid oxidation and *Escherichia coli* O157:H7 was investigated. TBA (thiobarbituric acid) formation was retarded by packaging of the meat with THY-H and EUG-MMT films. Although packaging process failed to completely inhibit the growth of *E. coli* O157:H7, significant (*P*<0.05) reductions in the population were provided. THY-H nanocomposite film was the most effective for the inhibition of *E. coli* O157:H7 growth along with the retardation of TBA formation. In conclusion, the results were promising revealing the use of the nanocomposite films containing MMT and H nanofillers doped with the natural plant extracts as packaging materials of fresh ground meat.

Key Words – Active meat packaging, Carvacrol, Eugenol, Halloysite, Montmorillonite, Thymol

- INTRODUCTION

Fresh meat products such as ground beef are commonly sold in markets at refrigerated temperatures. At these temperatures, lipid oxidation and microbial deterioration are the most critical factors which may result in undesirable changes in meat and meat products [1, 2]. Moreover, these foods may be contaminated with pathogenic bacteria such as *Listeria monocytogenes*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Escherichia coli* O157:H7 and *Yersinia enterolitica*, which are responsible for foodborne illnesses and deaths [3]. To overcome such problems, some prevention methods have been investigated. These methods include traditional packaging with oxygen permeable films, vacuum / modified atmosphere packaging (MAP), active/intelligent packaging and nanocomposite based packaging technologies [4].

Clays are naturally occurring nano-sized inorganic solid materials [5]. Incorporation of nanoclays in packaging materials provides them numerous benefits including improved barrier properties, chemical and thermal stability, retardation of flammability and easy recycling [6]. In addition to reinforcing packaging materials, nanoclays can provide active properties in the packaging materials, such as antimicrobial and antioxidant activity by means of being carriers of active materials [7]. Thymol (THY), eugenol (EUG) and carvacrol (CRV) are well-known natural phenolic compounds with GRAS (generally recognized as safe) status derived from aromatic plants and possess strong antibacterial activity against a wide range of pathogenic bacteria [8]. In this study, it was aimed to determine the contribution of novel active nanocomposite low density polyethylene (LDPE) films incorporated with nanoclays (montmorillonite (MMT) or halloysite (H)) and antibacterial plant derivatives (THY, EUG or CRV) to the inhibition of lipid oxidation and *E. coli* O157: H7 in ground beef.

- MATERIALS AND METHODS

Materials

Na^+ Montmorillonite (MMT) and halloysite (H) were purchased from Sigma (Sigma-Aldrich Chemicals Co., USA). Thymol (THY), eugenol (EUG), carvacrol (CRV), Tween 80, Thiobarbituric acid (TBA) and Butylhydroxyanisole (BHA) were provided from Merck, Germany. Linear low density polyethylene (LDPE) pellets was purchased from Rabigh Refining & Petrochemical Co. This LDPE has a density of 0.921 g/cm3, melt flow index of 1.1 g/10 min. and has a melting temperature of 122 °C. Fresh beef meat from a single carcass was bought from a local butcher in Kayseri, Turkey, and quickly transferred to laboratory and used in the experiments.

Escherichia coli O157:H7 ATCC 33150 was obtained from General Directorate of Protection and Control Vision, Kayseri, Turkey. Frozen *E. coli* O157:H7 stock culture was twice pre-activated in Nutrient Broth (Merck, Germany) at 37 ºC for 24h before use.

Preparation of Active Nanocomposite Films

3 g of solid MMT or H clays were dispersed in 100 mL of distilled water and stirred at 600 rpm for 5 min. Tween 80 was added to the mixture as an emulsifier at a level of 3% (w/v) and vigorously stirred for 24 h. Then the mixture was dried at 80 ºC for 8 h to evaporate the water. The solid material was finely milled to sizes less than 200 meshes using a planetary ball mill. Finally THY, EUG or CRV was incorporated with the resulting powder at the ratio of 5% (v/w).

Active nanocomposite films were produced by melt mixing in a twin screw co-rotating extruder (L/D = 42, *D* = 16 mm) using a temperature profile of 180, 175, 180, 175 and 180 ºC. LDPE and nanofillers containing the active compounds were fed to the vibrating hopper at the same time to obtain nanocomposite blends with 5% wt nanoclay. The resulting films were labeled as: (1) pure LDPE, (2) LDPE + CRV–MMT, (3) LDPE + EUG–MMT and (4) LDPE + THY–H.

Preparation and Packaging of Ground Meat

At first, beef meat was divided into 2 lots to carry out microbiological and TBA experiments. For preparation to microbiological analysis, beef was grounded using a sterile meat grinder (Tefal, China). Ground meat was sprinkle inoculated with the activated bacterial culture to obtain a targeted population of ~10^5 cfu/g. Then the contaminated meat was finely kneaded by hand for 5 min to provide homogeneous distribution of bacteria. 25 g of meat samples were weighed aseptically and packaged in active nanocomposite film materials (8cm x8 cm) and the packages were sealed. Control sample was packaged in pure LDPE film.
The beef which will be used for TBA measurements was grounded using the grinder described below and packaged in 25 g of portions in the active nanocomposite packaging materials. Control group was packaged in pure LDPE films.

Storage

All the samples were stored at 5 ºC for 7 days and evaluated at different times of storage, namely 0, 1, 4 and 7 days.

Enumeration of E. coli O157:H7

The package containing 25 g of the ground beef was carefully unsealed. The content of the package was transferred into a sterile stomacher bag and homogenized with 225 mL of sterile maximum recovery dilution (Merck, Germany) solution for 90s using a masticator. Then the serial dilutions were prepared and spread plated on SMAC Agar (Merck, Germany). The plates were incubated at 37 ºC for 24 h and forming colonies were counted. Results were expressed as log cfu/g.

Lipid Oxidation

Lipid oxidation of meats was evaluated by measuring TBA values according to perchloric acid method (4%) as outlined by Ulu [9]. Measured absorbance levels were multiplied by a factor 7.8 to obtain TBA values in mg malondialdehyde (MDA)/kg sample.

Statistical Analysis

Conventional statistical methods were used to calculate means and standard deviations. Collected data was subjected to statistical analysis using the SAS statistical software [10] with two-way allocation. Significant differences between the means were further analyzed using the Duncan's Multiple Range Test.

- RESULTS AND DISCUSSION

Figure 1 shows *E. coli* O157:H7 populations in the ground meats packaged and stored in active nanocomposite films. Inoculated level of the pathogen was 4.90 log cfu/g. Significant (P<0.05) and ongoing increases occurred in all samples during the storage. Bacterial population of the control sample packaged in pure LPDE film reached to 9.43 log cfu/g at the end of the storage. THY-H nanocomposite film held the bacterial numbers in the lowest level among the samples.

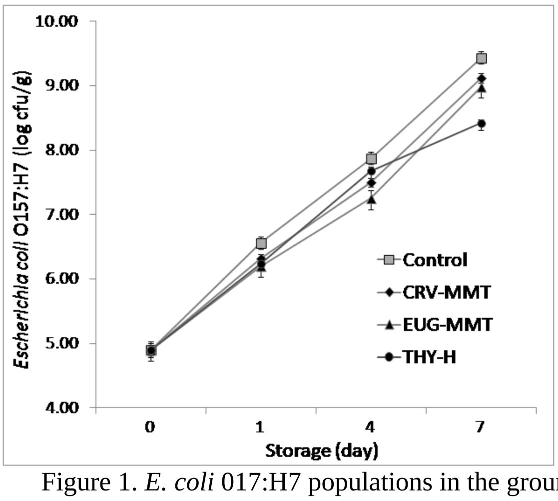

Figure 1. *E. coli* 017:H7 populations in the ground meats packaged in active nanocomposite films.

Lipid oxidation of the beef patty samples were followed by TBA measurements. Figure 2 illustrates the changes in TBA values of the samples during the refrigerated storage for 7 days.

Initial TBA value of the ground meat was 0.211 mg MDA/kg sample. Control sample and the sample packaged in CRV-MMT nanocomposite film exhibited high TBA levels at the end of the storage. EUG-MMT and THY-H films were quite effective to inhibit the TBA formation in the samples. TBA values of the samples packaged in those packaging films were 0.624 mg MDA/kg and 0.413 mg MDA/kg, respectively.

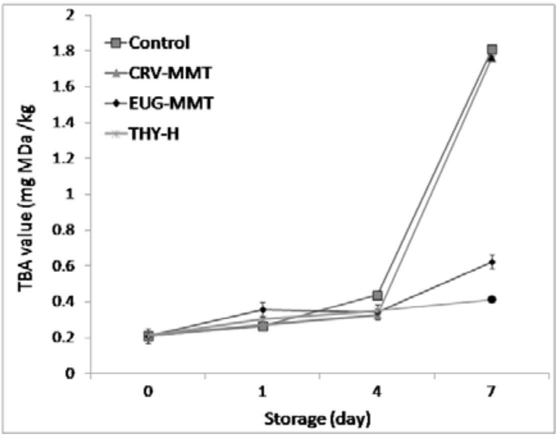

Figure 2. TBA values in the ground meats packaged in active nanocomposite films.

- CONCLUSION

Promising results were obtained related to use of the nanocomposite films containing MMT and H nanofillers doped with the natural plant extracts. *E. coli* O157:H7 population of the control meat packaged with pure LPDE film reached to high levels during the refrigerated storage. The highest TBA formation was also obtained in the same sample. THY-H nanocomposite film was the most effective one both as the inhibitor of *E. coli* O157:H7 growth and in the retardation of TBA formation. In conclusion, these nanocomposite packaging materials can be used in the maintaining of some quality parameters of the ground meats.

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