Innovative Polyamide-Based Packaging of Fresh Meat

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ABSTRACT: Innovative polymeric films based on blends of nylon 6 and ethylene-*co*-vinyl alcohol, previously tested by contact with the simulating fat-containing foods, were further tested as packaging for fresh minced meat. As quality attributes for the shelf-life evaluation, the microbiological parameters and some chemical–physical parameters were chosen. The changes that occurred during the evaluation period of 6 days were monitored by several means on both the polymer films and the fresh meat. The results suggest that the barrier properties of the polymeric films were shown to play a determinant role in the lipid oxidation, thus confirming the previous evaluation of the simulating foods.
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Key words: polyamide blends; barrier films; food contact; polymer characterization

INTRODUCTION

Nowadays the market distribution system of fresh meats (i.e., larger supermarkets and hypermarkets) requires a product that is able to maintain its original sensory and nutritional characteristics as long as possible.[1–3] The most important method to achieve this goal is represented by the use of the so-called cold chain, which means that the refrigeration and freeze is properly applied to inhibit the development of microbial flora originated from the meat deterioration and microbiological decay.[4] Although a natural antibacterial property exists in meals, the prevention of the microbial contamination remains an efficient way to limit its hygienic–sanitary and sensory damages. Some scrupulous hygienic norms during the slaughtering, transformation, and commercialization processes are not enough to prevent meat contamination. In fact, several studies have suggested that some methodologies for the conservation of fresh and transformed meats,[5,6,7] by using (1) preservatives; (2) bacterial cultivations with a specific antagonistic activity generating some specific metabolites (i.e., lactic acid and bacteriocins)[8]; and (3) gases, such as O_2, N_2, CO_2, or their gaseous mixtures; are able to hinder or delay the microbial increase.[9] Innovative polyamide 6 based polymer films were previously investigated with migration tests by using some fat simulating substances.[10] In the present work, we tested the said polymer films for a potential use in the packaging of the minced fresh bovine meats. The aim of this work was the to estimate the migration ability (particle cession) from the package to the produce and vice versa to ascertain their suitability into the alimentary field.[11–13] Furthermore, a comparison between the selected innovative polymer films and a commercially available polyethylene film is reported under general-purpose conditions used for fresh minced meat. Chemical–physical analyses and microbiological and technological tests were carried out on the meats before and after the packaging during the conservation period to estimate the ability of the investigated films to preserve the microbiological properties.

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EXPERIMENTAL

Materials

Polyamide 6 (Nylon) was kindly supplied by SNIA Tecnopolimeri coded F34L (η_{rel} = 3.4 at 20°C in sulfuric acid, density = 1.19 g/mL). Ethylene-*co*-vinyl alcohol (EVOH), containing about 29mol % of ethylene, was kindly supplied by Nippon Gohsey (density = 1.14 g/mL). The modified EVOH containing 2.4 wt % of grafted succinic anhydride (EVOH–COOH) was prepared according to a reported procedure.[14]

Blends preparation and film-blowing procedure

Blends, the composition of which is reported in Table I, were prepared according to a previously reported

TABLE I
Blend Compositions Used as Packaging Films with Commercially Available Meats

Polymer films	Codes	Composition (w/w)
Nylon 6	Ny6	100/0
Ny6/ethylene-*co*-vinyl alcohol blend	Ny/EVOH	75/25
Ny6/ethylene-*co*-vinyl alcohol/ethylene-*co*-vinyl alcohol carboxyl-modified blend	Ny/EVOH/COOH	75/20/5

procedure.[15] Films were prepared in a single-screw extruder and equipped with an annular die of 20 mm and a HAAKE take-off apparatus. Extrusion was performed at 20 rpm with a temperature profile of 250–250-250°C. The choice of the operating conditions was suggested by the bubble stability. Take-off speed was set at 4.5 m/min. The mean of the film thickness was 20 μm. In Table I, codes and compositions of the employed polymer films are reported.

Microbiological tests

The microbiological and chemical–physical analyses were carried out on commercially available minced meats before and after the packaging at 4°C in some polyamide 6 based films for a period of 6 days. A portion of the same meat was stored for comparison in a commercially available low-density polyethylene (LDPE) package under the same conditions. For the microbiological analyses in aseptic condition, 10 g of each meat sample was taken and homogenized in a stomacher Lab Blender model 400 (Seward, UK) for 2 min with 90 mL of sterile peptonated water (1 g/L peptone; 8.5 g/L sodium chloride); subsequent dilutions were obtained with the same diluent. The dilutions were plated onto specific culture media and incubated to the suitable temperatures and conditions, hereafter reported.

- Plate Count Agar (Oxoid): The plates were incubated for 48 h at 30°C to evaluate the total microbial content. MRS agar (Oxoid): The plates were incubated in anaerobiosis (Kit Anaerogen, Oxoid) for 72 h at 30°C, to isolate the lactic flora.
- Mannitol Salt Agar (Oxoid): The plates were incubated for 48 h at 30°C to isolate *Micrococcaceae*. YPD agar (yeast extract, 10g/L; peptone, 20 g/L; dextrose, 20 g/L; agar, 20 g/L): The plates were incubated for 5 days at 30°C to isolate the blastomycetic flora.
- Slanetz and Bartley (Oxoid): The plates were incubated for 48 h at 37°C, to identify *Enterococcus* spp. Violet Red Bile Glucose Agar (Oxoid): The plates were incubated for 48 h at 36°C, to isolate *Enterobacteriaceae*. Violet Red Bile Lactose Agar (Oxoid): the plates were incubated for 48 h at 44°C, to isolate fecal coliforms. After incubation, colonies were counted.

FTIR spectra

All spectra were carried out with a IFS 88 Bruker spectrometer purged with dry air and equipped with an MCT detector. Attenuated total reflectance FTIR spectra (ATR-FTIR) were measured by using a Specac horizontal ATR-equipped ZnSe crystal at 45° (six reflections), a resolution of 8 cm^{-1}, and at least 2000 scans. The spectra were corrected to compensate for the spectral band intensities due to the different penetration depths of the incident infrared radiation into the polymer films as described elsewhere.[16]

Color measurements of film

The color changes in the polymer film samples were carried out with a colorimeter model color sphere of Byk-Gardner, equipped with an integrating sphere having a diameter of 80 mm and a pulsed xenon flash lamp. The CIE Lab color system coordinates were calculated by measuring the reflectance spectra of polymer films by using an observer at 8° and D65 as illuminant (daylight).[17] The yellowness indices (YI) were calculated by reflectance spectra in the same manner as reported in ASTM D313/1996 and by using the corresponding untreated sample as reference.

Haze measurements of films

The transmittance of polymer films was measured with a colorimeter color sphere of Byk–Gardner in the transmission configuration by using a standard white reflection sample in the reflectance port and an opaque zero plate for calibration. The haze percent measurements (i.e., sample transmittance percent) were calculated as described in ASTM 1003 by using the corresponding untreated sample as reference.[18]

Measurements of meat's color changes

The measurements of the color changes of the fresh and conserved meats after 6 days in the various polymer packages were carried out by the following pro-

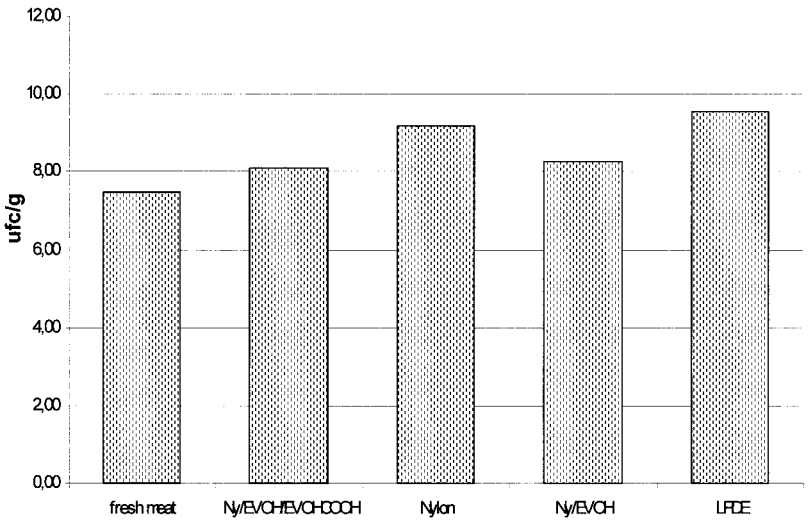

Figure 1 Development of microflora in fresh and packaged meat.

cedure: 20 mL of distilled water was added to 5 g of the meat sample and carefully dispersed by stirring for 5 min. After filtration with a Buckner funnel, the solution was subsequently split into two vials and centrifuged up to 13,000 rpm for 10 min. The obtained supernatant liquid fraction was used to record the UV–Vis spectra by means of a Varian spectrophotometer in a range of 250–750 nm.

RESULTS AND DISCUSSION

Microbiological tests

The results were obtained from the chemical–physical and microbiological analyses relative to the microbial population. The existence of the main bacteria groups and the pH values measured before and after the conservation of the meats are reported in Figures 1–3 for various polymer films. Figure 1 shows the total microbial concentration detected into the fresh and stored meats of the packages at 4°C for 6 days. The bacterial concentrations were found larger in both the meat stored into polyethylene and the pure nylon films than those detected in the fresh meat conserved into Ny/EVOH and Ny/EVOH/COOH polymer films. In Figure 2, the populations of the main microbial groups constituting the natural microflora and contaminants of the meat, such as lactic acid bacteria, *Micrococcaceae*, *Enterococcus* spp., yeast and molds, enterobacteria, and fecal choliforms, were reported before and after the packaging. This figure puts proves that the film made by the ternary blend is able to limit the increase of yeasts, *Micrococcaceae*, and the fecal choliforms, while the pure nylon sample is not able to perform any action of slowing down or inhibition of the existing bacterial flora, out of a slight reduction in the increase of *Enterococcus* spp. and the lactic acid bacteria. An intermediate behavior was observed for the meats stored in the Ny/EVOH blend film. On the contrary, the meats stored in the polyethylene film show an increase of all microbial groups up to 10^9 ufc/g, thus promoting the complete deterioration of the product.

The values of pH measured on the meats in various packages were reported in Figure 3. These data were collected before and after the conservation for a period of 6 days at 4°C. The pH of the meats stored in the Ny/EVOH/COOH film is lower than those compared with of the fresh meats. In contrast, the pronounced pH values in the meats stored in the nylon and polyethylene films clearly indicate the beginning of the deterioration processes. The meats in the binary Ny/EVOH alloy film shows an intermediate pH value with respect to other polymer materials based on Ny6 and Ny/EVOH/COOH films. The measured decrease of pH values into the stored meats in the Ny/EVOH/COOH film can be correlated with the increase of the lactic acid bacteria.[8] Therefore, these results suggest that the recorded pH values are in agreement with the trend of the mesophilic microbial population, as shown in Figure 1. These results also indicate that the composition of the ternary blend, imparting the best oxygen barrier,[10] shows an inhibition effect toward the development of the natural microflora. The measured surveying twofold activity suggests that the inhibition with respect to aerobic is dependent on the microorganisms and some microaerophilics, such as *Micrococcaceae*, yeasts, enterobacteria, and fecal choliforms. This result can explain the increase of the lactic acid bacteria, whose antagonistic effect toward the putrescent microflora is well documented.[9]

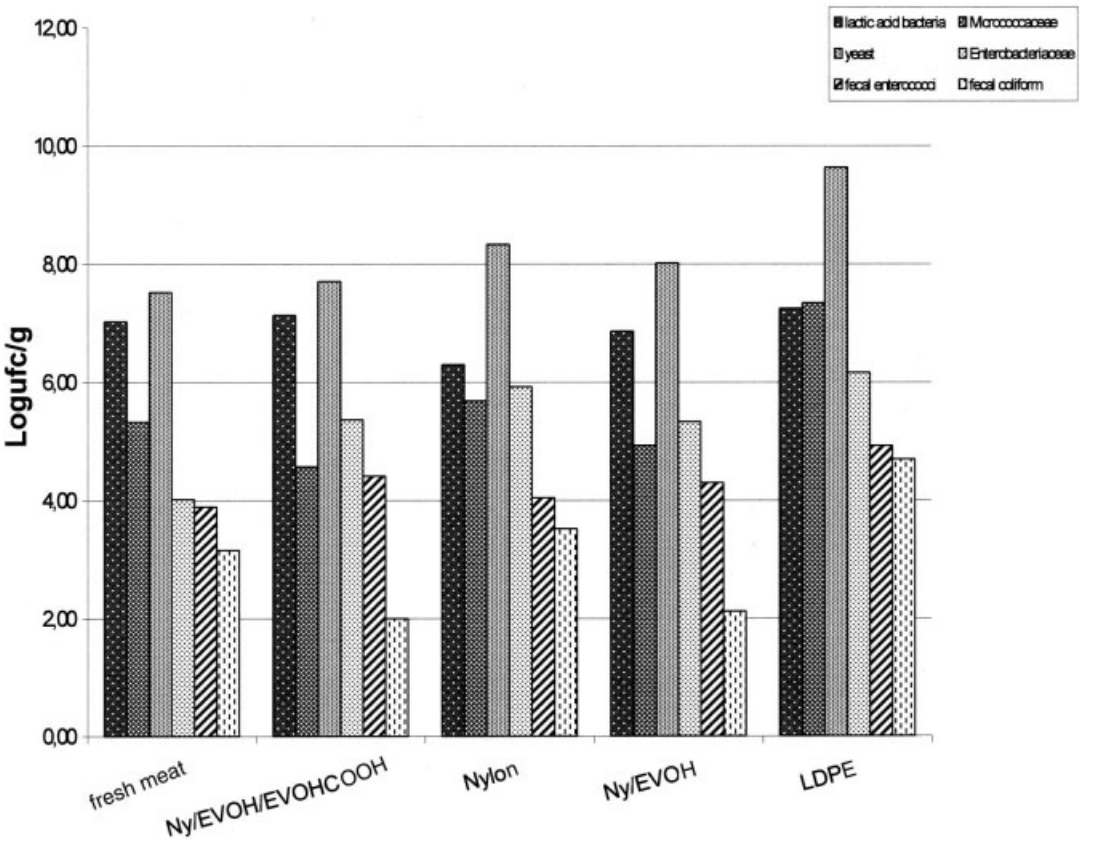

Figure 2 Development of spoilage microorganisms in fresh and preserved meat.

Meat color evaluation by means of spectrophotometric analysis

The deep-red color of the meat is a parameter of paramount importance, because it is chosen from the end-consumer as a freshness and quality index.[11] In fact, the chromatic changes in respect to the standard initial color are interpreted as signs of the bad conservation. The pigments of the meats are mainly constituted by the myoglobin, and in a minor content by the hemoglobin, cytochromes, and flavine. The myoglobin is a protein formed from both a globulinic and a prosthetic not polypeptidic component, defined as eme, showing a center directly linked to an iron cation. The eme shows a reductive or oxidative behavior depending on its ability to bind an oxygen atom, in this way determining the various chemical properties of

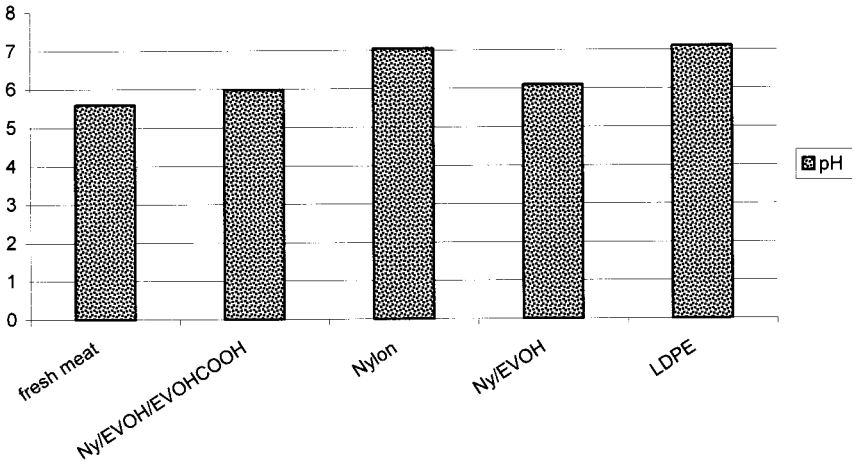

Figure 3 pH values of fresh and packaged meat.

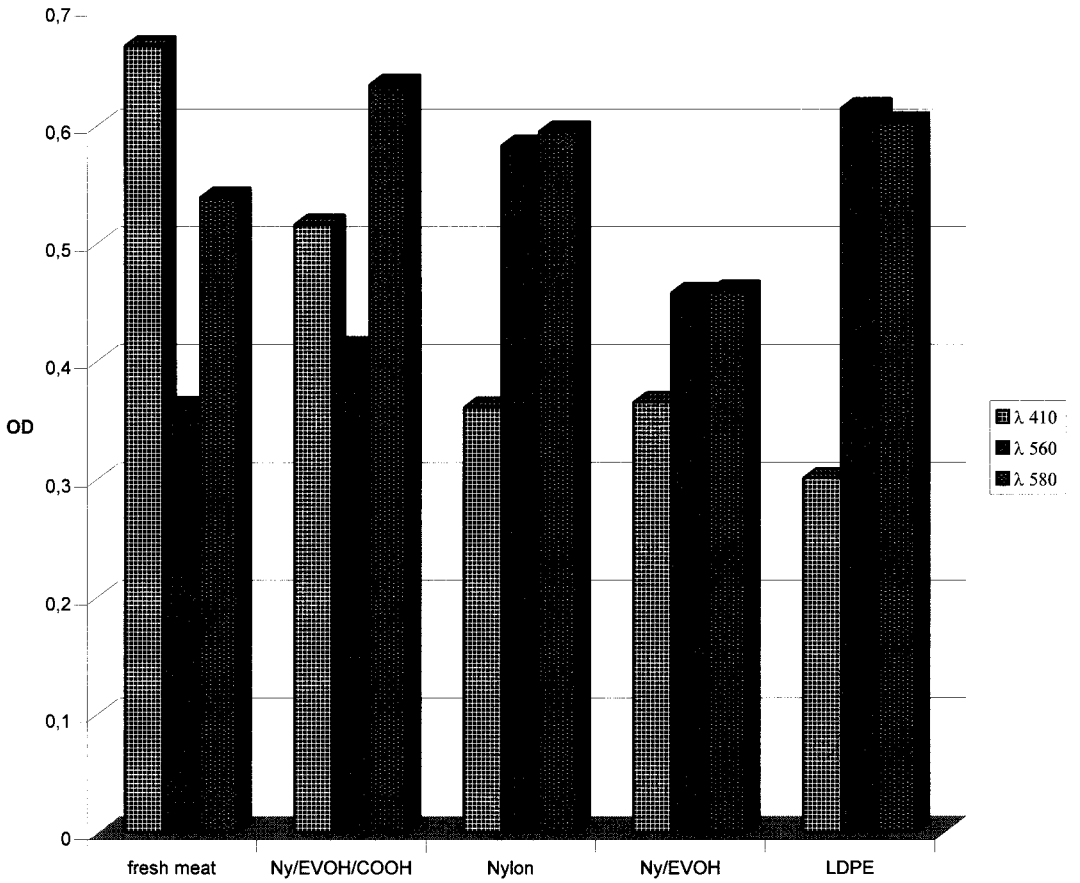

Figure 4 UV absorbances as function of preservation time.

the myoglobin. In a reduced form, iron(II) binds to an oxygen molecule imparting to meat a deep-red color (oxymyoglobin). On the contrary, iron(III) loses its ability to bind oxygen in the oxidized form and the meat becomes purple-brownish in color (metamyoglobin). We tried to determine color variations between fresh and stored meats by using the UV–Vis spectrophotometric measurements to follow the absorbance changes related to the concentration ratios between the oxymyoglobin and metamyoglobin contents. The absorbances are reported in Figure 4 at three different wavelengths, 410, 560, and 580 nm, for some different meats. The absorbance at 410 nm (corresponding to the myoglobin[12] of fresh meat) remains undoubtedly high when the meats are conserved into the ternary blend film packaging for 6 days. In contrast, in the same sample, the absorbances at 560 and 580 nm (corresponding to the oxidized state of myoglobin) are less pronounced[13] than those stored in the binary blend and LDPE films. These results are in agreement with the above-mentioned microbiological tests in which the ternary blend shows the best performances toward inhibition of the meat contamination, because the absorbance found at 410 nm is more pronounced than those films derived from the binary blend and pure polymer film.

Optical properties and spectroscopic characterization of the polymer films

The polymer film surface compositions of these polymer films nearby at the air/polymer interphase were previously reported by using attenuated total reflectance (ATR-FTIR) and photoacoustic (PA-FTIR) infrared spectroscopies.[10] Both infrared techniques can be used to collect data at the various surface depths near film surfaces.[16,20] The ATR-FTIR is often used to obtain a depth profile ranging from 0.1 to 0.3 μm of polymer film surfaces, whereas the PA-FTIR is complementarily applied to investigate thick regions reaching a depth penetration of 3–11 μm in a frequency range typically observed for the stretching vibration modes (1800–1500 cm^{-1}).[16] Some previous studies on polymer films were demonstrated that the nondestructive ATR-FTIR technique is a powerful tool to investigate on the compositional changes after the various surface treatments under certain degradative conditions.[21] The polymer films of Ny6, Ny/EVOH, and Ny/EVOH/COOH were conveniently investigated by using ATR-FTIR to explore in detail a thin penetration depth of the polymer film surfaces which remained in intimate contact with the minced meat at 4°C for 6 days. The measured ATR-FTIR spectra be-

fore and after the meat contact have been reported in Figure 5(a–c). According to our previous work,[10] the ATR-FTIR spectra give an insight into the distribution of the polymer components near the film surface, showing a prevalence of nylon 6 at the air/polymer interface. The film compositions appear to be independent of the meat treatment, because the intensity ratios between two predominant vibrations of Nylon 6 and EVOH components, found at 1650 and 1450 cm^{-1}, respectively, remain unchanged. Furthermore, no additional vibrations were detected after the meat treatment on the investigated film surfaces, indicating that the film contamination is negligible. It is worth noting that the ternary blend film, having two weak vibration bands at 1734 and 1710 cm^{-1} on the untreated film surface, shows the disappearance of the band found at 1710 cm^{-1} after contact with the fresh minced meat for 6 days. Because the carboxy-modified EVOH (EVOH-COOH) was obtained by the partial esterification of the grafted succinic moieties after processing,[14] these two bands are reasonably attributed to the existence of ester and acidic groups at the film surface derived from the anhydride ring opening reaction promoted by the hydroxyl groups contained in EVOH component in the ternary blend. On the basis of the above-reported pH changes observed in the packaged meats, we believe that the suggested initial deterioration could reasonably be explained with the subsequent disappearance of acidic forms at the meat/ternary blend film interface.

The optical properties, such as haze percent (i.e., related to the percentage of transmittance) and color changes, were also measured under the same packaging polymer films. The haze percent and color changes measured for the Nylon 6 and binary and ternary polymer blend films are reported in Table II after an intimate contact with the minced meat. The results indicate that no appreciable changes ($\Delta E < 1$) were detected for both the color and the percent haze of these films. The color analysis is very sensitive to detect the existence of the meat contaminants on the film surface, when the daylight, by using D65 as illuminant, is applied for these measurements. The existence of the meat contamination or a possible swelling phenomena, derived from the water containing food and meat residues, can reasonably increase the scattered light at the investigated polymer film surfaces. The haze percent retention of the polymer films suggests that their morphology is maintained under the packaging conditions.[22] The results obtained for the ternary blend film, showing the best retention of haze percent and color, are in agreement with the above reported measurements on the packaged meat.

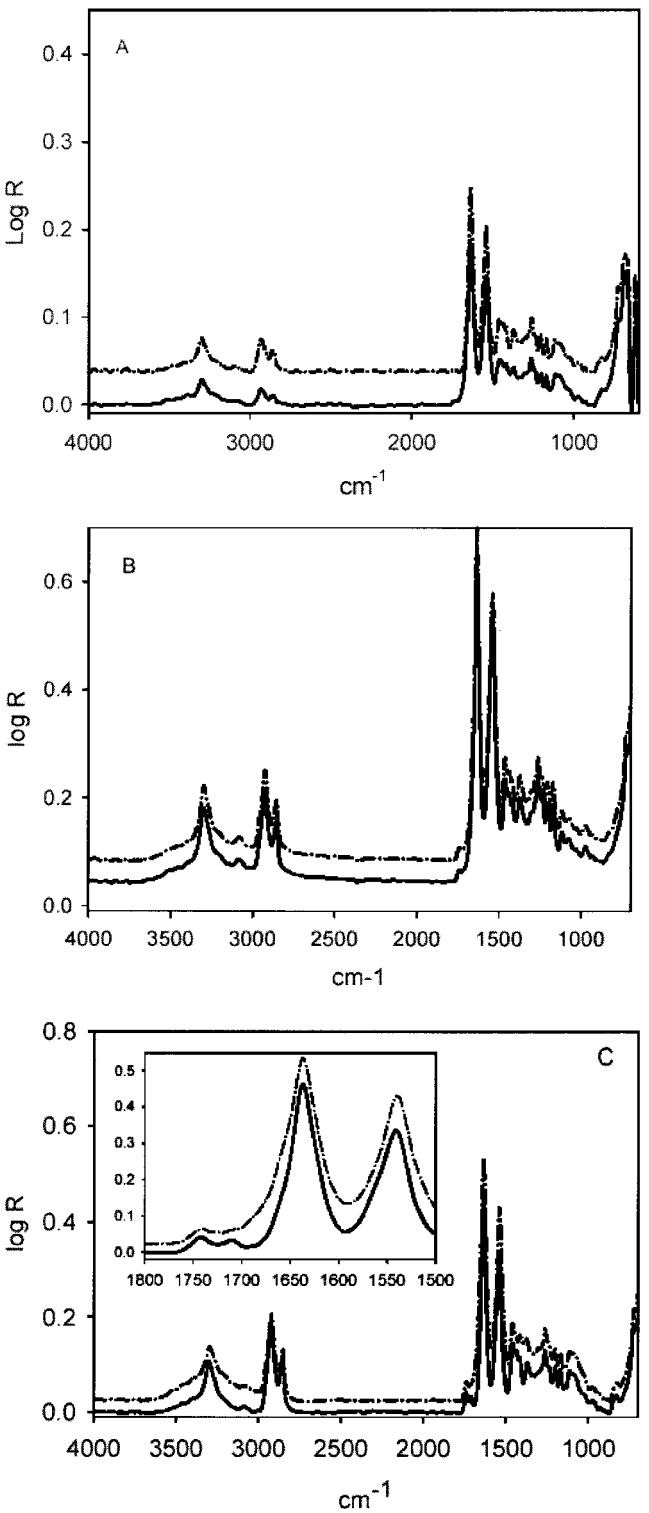

Figure 5 (a) FTIR-ATR spectrum of Ny6 polymer film before (—) and after (–·–) an intimate contact with minced meat for 6 days at 4°C. (b) FTIR-ATR spectrum of the Ny6/EVOH binary blend film before (—) and after (–·–) an intimate contact with minced meat for 6 days at 4°C. (c) FTIR-ATR spectrum of the Ny6/EVOH/COOH ternary blend film before (—) and after (–·–) an intimate contact with minced meat for 6 days at 4°C.

TABLE II
Color and Haze Changes of the Investigated Polymer Films as a Function of the Treatment Time with a Commercially Available Meat

Samples[a]	Color[b] ΔE (D65)	Haze[b] (%)
Ny6	0.40 ± 0.02	11.0 ± 0.02
Ny/EVOH	0.28 ± 0.02	13.2 ± 0.02
Ny/EVOH/COOH	0.11 ± 0.02	5.0 ± 0.03

[a] The untreated polymer materials were used as reference for color and Haze measurements.
[b] Treated films were put in contact with meat for 6 days at 4°C.

CONCLUSION

We have demonstrated that an innovative film based on Ny/EVOH/modified EVOH ternary blend film, in comparison with the pure Nylon 6 and a binary Ny/EVOH blend film, shows a good oxygen barrier inhibiting the oxidation of commercially available fresh minced meat. The same behavior was observed for the same investigated polymer materials tested with some fat-containing simulating foods. Under the same testing conditions, the enzymatic and fermentative processes, due to the meat deterioration, are simultaneously decreased when a ternary blend film is used. These inhibition effects were attributed to the blend component intimate dispersion into achieving an improvement of the EVOH atmospheric oxygen barrier of the polymer film matrix. The retention of the daylight transmittance and color of the tested polymer films maintained an intimate contact with the minced meat shows the absence of the contamination and swelling phenomena under the packaging conditions.

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