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Application of Antimicrobial Nanocomposites in Ready to Eat Meat Products

Zehra Ayhan [1 +], Donatella Duraccio [2], Birgül Özcan [3], Okan Eştürk [1], Serra Nalçabasmaz [1], Clara Silvestre [2], Sossio Cimmino [2], Gülsüm Erol [1], Murat Altan [1], Bengisu Toplu [1]

[1] Mustafa Kemal University Food Engineering Department Hatay Turkey
[2] Istituto di Chimica e Tecnologia dei Polimeri-CNR, Pozzuoli Naples Italy
[3] Mustafa Kemal University Biology Department Hatay Turkey

Abstract. The aim of this work was to explore preparation, characterization and applications of active nanomaterials for food packaging. Nano-iPP film and active-nano-iPP film were prepared with the addition of 1% of nanoclay, and with 1% nanoclay plus 5% poly-β-pinene (PβP), respectively. OTR and WVTR and antimicrobial properties of the films were determined. While the addition of 1% nanoclay reduced OTR and WVTR by 10% and 24% comparing to neat iPP, addition of 1% nanoclay and 5% PβP reduced OTR and WVTR by 24% and 31%, respectively. The WVTRs of iPP, iPP-nanoclay, iPP-nanoclay-PβP were 1.88, 1.43 and 1.30 g m^{-2} day^{-1}, respectively. The material containing PβP showed antibacterial effect. Sliced salami was packaged using these nanomaterials and multilayer material (control) under air, vacuum and 50% CO_2-50% N_2 and cold stored at 4°C. Results showed that the best results were obtained in vacuum and high CO_2 applications using multilayer material with the product shelf-life of 75 days. The shelf-life of the sliced salami was 50 days for nanomaterial containing PβP under vacuum; however, it was limited to 30 days under high CO_2 MAP application since the active material was more effective when it is in direct contact with the food.

Keywords: isotactic polypropylene, poly-β-pinene, nanoclay, nanomaterial, active packaging, food packaging, ready-to-eat food products, modified atmosphere packaging

1. Introduction

Nowadays, multilayered packaging materials with low gas permeability are extensively used in ready to eat meat products to assure food quality, safety and required shelf life. However, due to complex production techniques, high cost and non-recyclable nature of the multilayered materials, there is a need for new-one layer materials with improved barrier properties using nanotechnology as an alternative to multilayered materials. The objectives of this work were to prepare iPP/organophilic clay and iPP/Poly-β-pinene/clay nanocomposites, to determine permeability and antimicrobial properties of the produced films and to apply these films in food packaging and investigate the effects of these nanomaterials and vacuum/modified atmosphere packaging technology on the microbial quality and safety and shelf life of ready to eat meat products. This study will provide if new nanomaterials with increased barrier properties and antimicrobial activities could be used as an alternative to multilayered packaging materials for meat products to extend the shelf.

2. Materials and Methods

2.1. Materials

+ Corresponding author. Tel.: +90 326 2455845 (1055); fax: +90 326 2455832.
E-mail address: zehra.ayhan@gmail.com.

Three materials are produced and characterized in this study: mono iPP (control) (PPR 3221), nano-iPP film containing 1% nanoclay (Dellite® 67G) and active-nano-iPP film containing 1% nanoclay and 5% poly-β-pinene (Piccolyte® S115). The commercial multilayer material (PP/PA/EVOH/PE, Superfilm, Gaziantep, Turkey) was used as a control in food packaging trial.

2.2. Preparation of Materials

Components were mixed and processed through twin screw extruder (Collin Teach-Line Twin screw kneader ZK25T/SCD15, Dr. Collin GMBH, Ebersberg, Germany) and converted into pellets by using pelletizer (Collin Teach-Line Pelletizer171T, Dr. Collin GMBH, Ebersberg, Germany). The pellets were processed into a single screw extruder (Collin Teach-Line Extruder E20T/SCR15, Dr. Collin GMBH, Ebersberg, Germany) for obtaining films by a Collin Teach-Line Chill Roll CR72T (Dr. Collin GMBH, Ebersberg, Germany).

2.3. Characterization of Materials

2.3.1. Oxygen Transmission Rate (OTR) and Water Vapor Transmission Rate (WVTR)

OTR (ASTM D3985) and WVTR (ASTM E96) were determined by using a Multiperm ExtraSolution (Pisa, Italy). The area of the film involved was 50 cm^2. OTR was performed at 23 °C and 0% RH. The WVTR was performed at 38 °C and 90% RH. Three measurements were performed for each composition on different film.

2.3.2. Antimicrobial Activity of Materials

Antimicrobial activity of PβP containing nanomaterial was tested using ASTM method (E2149-10) [1] on test organisms of *E. coli 25922*. The results are presented in either percent reduction when measuring CFU/ml or Log_{10} bacterial reduction when calculating mean log10 density of bacteria as follows;

Reduction, % (CFU ml^{-1}) = (B-A/B) × 100

Log_{10} reduction= Log_{10} (B)−Log_{10} (A) where;

A = CFU ml^{-1} for the flask containing materials (PβP containing nanomaterial)

B = CFU ml^{-1} the flask containing control material (material containing only nanoclay)

2.4. Food Packaging Trial

Salami was obtained from producer (Maret, Turkey) a day prior to processing and packaging and sliced in the thickness of 2.5 mm with the slicing equipment (Scharfen es 300, Germany) under hygienic conditions. 200 g of sliced salami was packaged in 3 different bags (nanomaterial-M1, active-nanomaterial-M2 and multilayered material-M3) under 3 different atmospheres (air-MAP1, 50%CO_2 and 50%N_2-MAP2 and vacuum-MAP3) using packaging system (Reepack rv 300, Italy). Packaged salami was stored at 4 °C and 50% RH for 90 days. Headspace gas analysis and microbiological analysis were conducted during storage. The study was triplicated.

2.5. Headspace Gas Analysis

Oxygen and carbon dioxide concentrations (%) in the headspace of the packages were determined using a gas analyser (PBI Dansensor, Ringsted, Denmark). Gas analysis was performed by inserting a needle attached to the gas analyser through an adhesive seal fixed on the top cover and gaseous sample was extracted from the headspace using an airtight syringe attached to the analyser.

2.6. Microbiological Analysis

The packaged products were scanned for pathogens (*Salmonella* spp. and *Listeria monocytogenes*) by VIDAS technique (enzyme based floresan technique) right after the processing on the day 0 [2], [3]. The total mesophilic aerobic bacteria and yeast and molds were monitored during the storage period using FDA-BAM methods [4], [5]. The results were interpreted based on the Turkish Food Codex.

3. Results and Discussion

3.1. Material Properties

The addition of 1% nanoclay reduced the OTR approximately 10% comparing to neat iPP. The OTR was significantly reduced by 24% with addition of both 1% nanoclay and 5% PβP. The addition of 1% nanoclay or 1% nanoclay + 5% PβP reduced WVTR by 24% and 31%, respectively in comparison to plain iPP. Addition of clay+poly-β-pinene was more effective on reducing WVTR than that of OTR (Table 1).

Table 1. Properties of the packaging films.

Material type	**Thickness (μm)**	**OTR (cm^3 m^{-2} day^{-1})**	**WVTR (g m^{-2} day^{-1})**
iPP	93	1410	1.88
iPP-nanoclay	89	1282	1.43
iPP-nanoclay-PβP	93	1061	1.30
PP/PA/EVOH/PE	115	2	4

Nanomaterial containing PβP reduced the test microorganisms 1.33 log CFU ml^{-1} (or 24.3% reduction) comparing to the control. This result showed that the material containing PβP had antibacterial effect. Since PβP is immobilized antimicrobial agent and does not diffuse from the material, there is direct contact of the material containing PβP needed for antibacterial effect.

3.2. Headspace Gas Composition

O_2 and CO_2% in the headspace of salami packages were presented by Fig. 1 & 2, respectively. The gas composition was only measured in MAP packages. O_2% was 21% in MAP1 at day 0 for M1 (iPP-nanoclay) and M2 (iPP-nanoclay-PβP) and stayed unchanged for 50 days of storage. Although there was not much change in oxygen for M1-MAP1 during 90 days, O_2% dropped to 1% for M2-MAP1 after 50 days of storage possibly due to extensive yeast and mold growth. The oxygen for M3-MAP1 (vacuum packaging with multilayer material) decreased during increased storage. This reduction was possibly due to the consumption of oxygen by the product for chemical or microbial activity since the OTR of the material was quite low. For M1-MAP2 (50% CO_2 and 50% N_2) and M2-MAP2, the oxygen was 0% in the day 0 and it reached to 19-20% after 10 days of storage and remained at this level for the rest of the storage which is attributed to high OTRs of the materials. However, the O_2% in M3-MAP2 application was less than 1% during storage due to low OTR of multilayer material.

The CO_2% in M1-MAP1 was 0% in the beginning and reached to 1.53% at the end of the storage. The CO_2% in M2-MAP1 was under 1% during 50 days of storage and reached to 5-6% after 50 days. This increase was possibly due to microbial growth since there were visible mold colonies on the product surfaces after 60 days of storage. For MAP2 application of M1 and M2, the CO_2% started with 50% and rapidly depleted during storage and remained less than 1% after 10 days of storage. This reduction might be due to both solubility of CO_2 in the food products with high fat content and high CO_2TR of these materials. For M3-MAP1, CO_2 started with 0% and reached to 3.6% on the 90 th days of storage. The increase in CO_2 in this application might be attributed to microbial activity when the reduction of oxygen level was also considered during storage. For M3-MAP2, the beginning level of 50% CO_2 reduced to 11% in the 5 th day of storage and stayed stable for the rest of the storage. This rapid decrease in CO_2 could be explained by the solubility of this gas in the sliced salami since the permeability was quite low for the multilayer material.

3.3. Microbiological Quality

The food relevant pathogens (*Salmonella* spp. and *Listeria monocytogenes*) were not detected in the products right after the processing and packaging operations. According to the Turkish Food Codex, these two pathogens are not allowed to present in heat treated meat products like salami.

The total aerobic mesophilic bacterial and yeast and mold counts were determined during the entire storage (data not shown). There was no bacterial growth at all applications for the first 20-30 days of storage which is possibly due to chemical additives (nitrites) in the product, however, for the extended period of storage, the differences in the bacterial growth was related to antimicrobial effect of PβP and CO_2. The PβP containing material was more effective on bacteria than yeast and molds. PβP containing nanomaterial was

more effective in vacuum application due to direct contact with food surface and there was no bacterial growth during 75 th day of storage. There was no bacterial growth observed in M1-MAP3 application during 40 th day of storage and in M3-MAP3 during 60 th day of storage. There was also no bacterial growth during 50 days of storage in M3-MAP2 probably due to antimicrobial effect of CO_2. For the application of air atmosphere, bacterial growth started in the 30 day of storage and increased for the rest of the storage. In general, the total bacterial load under vacuum for all materials was 1-2 log CFU g^{-1} less than that of MAP applications.

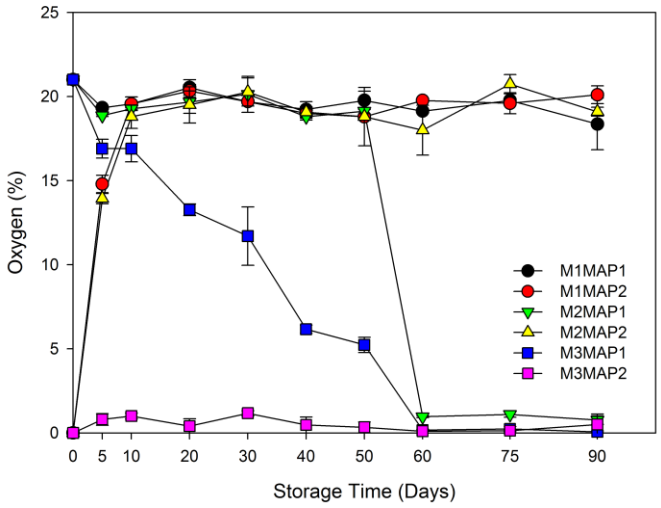

Fig. 1. Headspace O_2% during storage (M1: iPP/nanoclay, M2: iPP/nanoclay/PβP, M3: PP/PA/EVOH/PE, MAP1 (air): 21% O_2-79% N_2, MAP2:50% CO_2-50% N_2, MAP3: vacuum)

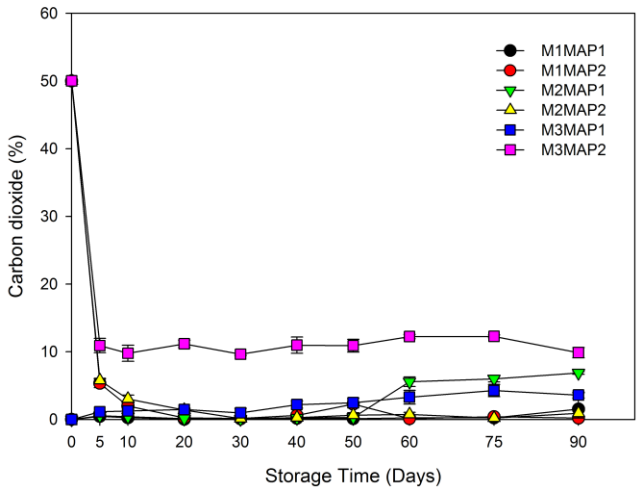

Fig. 2. Headspace CO_2% during storage (M1: iPP/nanoclay, M2: iPP/nanoclay/PβP, M3: PP/PA/EVOH/PE, MAP1 (air): 21% O_2-79% N_2, MAP2:50% CO_2-50% N_2, MAP3: vacuum)

There was no yeast and mold growth in the first 30 days of storage in all applications except M1-MAP2. No yeast and mold growth observed for M3-MAP3 during entire storage of 90 days and the bacterial growth started after 60 days of storage. Sliced salami packaged with M2-MAP3 had no yeast and mold growth during 50 days of storage, however, there was very rapid growth after 50 days and the total load reached to 6.62 log CFU g^{-1} at the end of the storage.

There were no limits defined for total bacterial count and yeast and mold counts in the Turkish codex [6] for heat treated meat products. However, the maximum total yeast and mold counts were limited to 103 CFU

g^{-1} in the previous codex [7]. This limit and also sensory evaluation were considered when the shelf life was suggested for each application.

Results showed that the best results were obtained in vacuum and high CO_2 applications using multilayer material and the shelf lives were suggested as 75 days for these two applications. The shelf life of the sliced salami was 50 days for nanomaterial containing PβP under vacuum; however, it was limited to 30 days under high CO_2 MAP application since the active material was more effective when it is in direct contact with the food in the case of vacuum.

4. Acknowledgements

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