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BIODEGRADABLE ACTIVE PACKAGING APPLICATION ON FRESH MINCED BEEF

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Abstract

Meat quality and safety is of great importance, and it frequently depends on the packaging technology. Recently, active packaging gained more and more attention in food industry, due to its ability to carry antimicrobial and antioxidant ingredients, which could lead to enhanced properties of the packed food. The aim of this study was to determine the quality parameters of fresh minced beef during storage in the presence of an active packaging material based on PLA, PHBV and nano emulsion of nisin and dill essential oil. Physical-chemical and microbiological analysis were performed for fresh minced beef quality determination. Furthermore, a challenge test was performed using Escherichia coli ATCC 8739 as test microorganism to determine the developed materials antimicrobial efficacy. The results showed that the application of active packaging decreased the microbial load during storage at 4°C.

Key words*: active packaging, essential oil, food safety, minced beef, nisin.*

INTRODUCTION

Food safety represents a very important factor during storage, especially the microbiological safety of the products. Fresh meat is generally categorized as a highly perishable food product, acting as a great environment for microorganisms' growth, therefore being susceptible to microbial spoilage as the main cause of deterioration (Cercel et al., 2017; Şimşek et al., 2017). Due to the negative effects that spoilage can have on consumers (health issues) but also economical loss for producers there is an increasing need to develop new techniques to maintain fresh meat quality. One of these techniques is represented by active packaging development with antimicrobial properties. As a general requirement from both food manufacturers and consumers for natural, safe and minimally processed foods a group of antimicrobial agents has been often used, represented by antimicrobial peptides which present various properties such as antifungal, antibacterial and antiviral properties (Bahrami et al., 2019). These peptides can be incorporated into films, coatings and food matrices, having a wide possibility of use within food industry. One of the most used peptides, especially in meat industry is nisin, which is a bacteriocin belonging to the lantibiotic class, produced by *Lactococcus lactis* subsp. *lactis*. It is composed of 34 amino acids and has a molecular mass of 3.5 kDa (Leelaphiwat et al., 2022). It has been recognised as safe (GRAS) by both Food and drug Administration (FDA) (Santos et al., 2018; Jia et al., 2021) and also approved by the Food Standards Australia New Zealand and the European Food Safety Authority (Gedarawatte et al., 2021). Furthermore, nisin possess great antimicrobial activity against a great variety of Gram-positive bacteria (Cui et al., 2016; Martillanes et al., 2021; Martinez-Rios et al., 2021). Bacteriocins incorporation in polymeric food packaging has been intensively studied in the past years due to their great ability to interact with the external surface of the microorganisms, not being necessary the internalization of the bacteriocin in order to fulfil its role (Santos et al., 2018). Therefore, application of nisin in packaging materials was studied using as base polymers like chitosan

(Divsalar et al., 2018; Remedio et al., 2019; Cao et al., 2019), cellulose (Wu et al., 2018), sugarcane bagasse (Yang et al., 2020), polyvinyl alcohol (Wang et al., 2015; Settier-Ramírez et al., 2021), pullulan (Hassan & Cutter, 2020) or polylactic acid (PLA) (Gulzar et al., 2022; Arias et al., 2022).
The aim of the present study was to assess the quality parameters of fresh minced beef which was stored at refrigeration temperatures (4±0.5°C) in the presence of a newly developed active packaging material based on PLA, poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and nano emulsion of nisin and dill essential oil. The antimicrobial efficiency of the developed packaging materials was also tested using a challenge test with *Escherichia coli* ATCC 8739 as test microorganism.

MATERIALS AND METHODS

Materials

In this experiment (Figure 1), beef samples kept in the presence of films obtained by electrospinning (ES), after different periods of storage at refrigeration temperatures (4±0.5 °C), were analysed from a physical-chemical and microbiological point of view.
The tested materials were obtained by deposing on the surface of PLA and PHBV films (using electrospinning technique) of a nanoemulsion formed of dill essential oil (Dill EO) and nisin as antimicrobial agents.
The fresh beef purchased from a local butchery in Bucharest, Romania, was processed by cutting and mincing and packaged as follows:
I. 100 g beef packed in a PET tray (L=8 cm, l=8 cm, h=4 cm), in contact with the PLA film obtained by ES (circular form) which was placed at the base of the tray, in direct contact with the sample.
II. 100 g beef packed in a PET tray (L=8 cm, l=8 cm, h=4 cm), in contact with the PHBV film obtained by ES (circular form) which was placed at the base of the tray, in direct contact with the sample.
III. 100 g beef packed in a PET tray (L=8 cm, l=8 cm, h=4 cm), considered control sample.
After packaging, the samples were stored at 4±0.5°C, to determine the shelf life of minced fresh beef. In Table 1 the samples and their coding are presented. As the first moment of analysis (Day 0), fresh minced beef sample was analysed (before packaging).

Table 1. Coding and sample description

Sample code	Description
Beef control	Minced beef sample packed in a PET casserole, considered the control sample and stored at 4°C
Beef PLA/Nisin/Dill EO	The minced beef sample packed in the PET casserole in contact with the PLA film obtained by ES and stored at 4°C
Beef PHBV/Nisin/Dill EO	The minced beef sample packed in the PET casserole in contact with the PHBV film obtained by ES and stored at 4°C

Minced beef | Minced beef sample packaged in the presence of the test material

Figure 1. Experimental design

Methods

Further, during the refrigeration period, the following physical-chemical analyses were performed: pH determination; determination of free acidity; determination of dry matter; determination of the a_w index; determination of free ammonia (freshness analysis), determination of colour and also microbiological analysis such as determination of total viable count (TVC), *Enterobacteriaceae* and *E. Coli*/Coliforms.
Briefly, the pH determination was performed using a pH meter WTW INOLAB 720 series type with automatic temperature compensator. Free acidity was determined by titration with NaOH 0.1 N in the presence of phenolphthalein as indicator. Dry matter content (DM) was performed by weighing 5 g of sample, which was further subjected to drying at 105°C using a RADWAG MAC 50 thermobalance. The results were expressed as a percentage (%). Water activity index (a_w) of the tested samples was determined using a NOVASINA equipment by introducing the sample into specific recipients of the equipment and the

value of a_w was read when stable at 25°C. Free ammonia was determined using the Nessler reagent. Colour determination was performed at room temperature using a HunterLab colorimeter, Miniscan XE Plus.

RESULTS AND DISCUSSIONS

Regarding the pH values (Table 2) of the minced beef, it can be observed that they did not vary significantly compared to the sample analysed on the day of packaging (Day 0 of the analysis). The acidity of the minced beef samples decreased during the storage period, indicating the beginning of degradation of the samples.

However, the Beef PHBV/Nisin sample presented the highest acidity value after five days of refrigeration, compared to the other two studied samples. In Table 3, the values of dry matter and humidity of samples are presented. No significant changes were observed in the values obtained for moisture and dry matter for the analysed samples.

Table 2. Evolution of pH and free acidity during the refrigeration storage

Sample / Moment of analysis	pH			Acidity (oleic acid/100 g)		
	Day 0	Day 3	Day 5	Day 0	Day 3	Day 5
Beef control	5.77 ± 0.014	5.75 ± 0.183	5.68 ± 0.035	8.315 ± 1.378	5.345 ± 0.318	5.065 ± 0.261
Beef PLA/Nisin/Dill EO	5.77 ± 0.014	5.71 ± 0.134	5.69 ± 0.021	8.315 ± 1.378	5.725 ± 0.233	5.255 ± 0.063
Beef PHBV/Nisin/Dill EO	5.77 ± 0.014	5.68 ± 0.028	5.75 ± 0.028	8.315 ± 1.378	5.645 ± 0.615	5.400 ± 0.056

Table 3. The values of dry matter and moisture determined during the refrigeration storage

Sample / Moment of analysis	Day 0		Day 3		Day 5	
	Humidity%	DM%	Humidity%	DM%	Humidity%	DM%
Beef control	76.518 ± 0.449	23.482 ± 0.449	76.841 ± 0.424	22.159 ± 0.424	76.597 ± 0.347	23.403 ± 0.347
Beef PLA/Nisin/Dill EO	76.518 ± 0.449	23.482 ± 0.449	76.700 ± 0.283	23.299 ± 0.283	76.682 ± 0.101	23.318 ± 0.101
Beef PHBV/Nisin/Dill EO	76.518 ± 0.449	23.482 ± 0.449	73.373 ± 4.927	26.626 ± 4.927	76.738 ± 0.376	23.261 ± 0.376

Table 4. The a_w index values determined during the refrigeration storage

Sample / Moment of analysis	Day 0	Day 3	Day 5
Beef control	0.980 ± 0.002	0.980 ± 0.000	0.985 ± 0.001
Beef PLA/Nisin/Dill EO	0.980 ± 0.002	0.971 ± 0.002	0.979 ± 0.000
Beef PHBV/Nisin/Dill EO	0.980 ± 0.002	0.975 ± 0.002	0.979 ± 0.000

Following the analysis of the data in Table 4, a decrease tendency of the a_w index can be observed for the meat samples packed in the presence of PLA/Nisin and PHBV/Nisin films and stored in a refrigerated state at a temperature of 4±0.5°C, compared to the Control sample. This leads to the conclusion that the water available for the development of microorganisms has decreased, thus preventing the alteration of the product from a microbiological point of view.

Table 5. The NH_3 content of minced beef during refrigeration storage

Sample / Moment of analysis	Day 0	Day 3	Day 5
	NH_3		
Beef control	Negative	Negative	Weakly positive
Beef PLA/Nisin/Dill EO	Negative	Negative	Negative
Beef PHBV/Nisin/Dill EO	Negative	Negative	Negative

The content of NH_3 is directly related to the freshness assessment of the meat. The results of the analyses regarding free ammonia (Table 5) show that the minced beef sample packed in the presence of the studied films and stored at 4±0.5°C did not show signs of the beginning of product degradation on day 5 of storage, compared to the control sample.

Regarding the colour of the minced beef samples, a decrease of the values of L*, a* and b* parameters was observed for all the studied samples during the refrigerated storage period, compared to the initially analysed sample (Table 6), the colour of the samples being modified on the last day of the analysis (Figure 2).

Table 6. The values of the L*, a* and b* parameters for the tested samples during refrigeration storage

Moment of analysis / Sample	Beef control			Beef PLA/Nisin/Dill EO			Beef PHBV/Nisin/Dill EO		
	L*	**a***	**b***	**L***	**a***	**b***	**L***	**a***	**b***
Day 0	39.84 ± 0.65	22.89 ± 0.79	21.00 ± 0.37	39.84 ± 0.65	22.89 ± 0.79	21.00 ± 0.37	39.84 ± 0.65	22.89 ± 0.79	21.00 ± 0.37
Day 3	35.77 ± 0.48	14.3 ± 0.80	16.08 ± 0.22	39.25 ± 1.16	11.78 ± 0.76	15.53 ± 0.32	35.04 ± 0.65	11.20 ± 0.72	15.41 ± 0.22
Day 5	40.28 ± 1.46	17.28 ± 0.46	18.39 ± 0.25	39.04 ± 0.67	11.22 ± 1.21	16.22 ± 0.32	35.14 ± 0.23	14.29 ± 1.15	16.94 ± 0.52

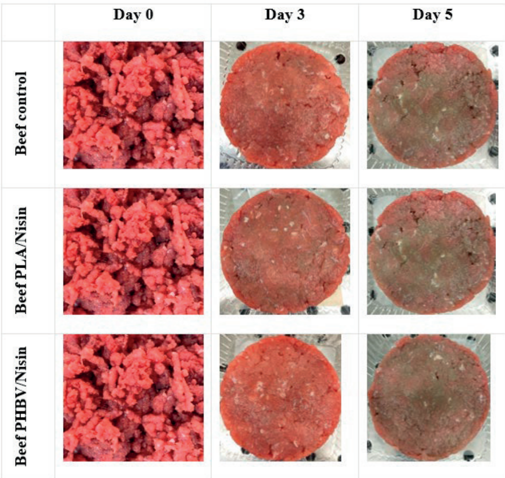

Figure 2. Appearance of minced beef samples during refrigerated storage

Regarding the microbiological analysis, the evolution of TVC values, as presented in Table 7, is of significant increase for the Beef control and Beef PHBV/Nisin samples, namely by approximately 2 logarithmic cycles, while for the Beef PLA/Nisin sample there was an increase of approximately 1 logarithmic cycle, during the refrigeration storage.

Further, in Table 8 it can be observed the presence of both *Enterobacteriaceae* and *E. coli*/Coliforms in all analysed samples. After 3 days of refrigeration storage of the packed minced beef in the presence of the two tested films, no more *Enterobacteriaceae* colony forming units (CFU) were identified in the samples compared to the control sample, proving the antimicrobial efficiency of the two tested films.

Table 7. TVC values of the tested beef samples

Sample / Moment of analysis	**Day 0**	**Day 3**	**Day 5**
	Total viable count (lgCFU)		
Beef control	2.26	3.69	4.42
Beef PLA/Nisin/Dill EO	2.26	3.03	3.82
Beef PHBV/Nisin/Dill EO	2.26	3.27	4.26

Table 8. The values obtained following the determination of *E. Coli* / Coliforms and *Enterobacteriaceae*

Sample / Moment of analysis	Day 0	Day 3	Day 5	Day 0	Day 3	Day 5
	*Enterobacteriaceae**			*E. Coli* / Coliforms*		
Beef control	+	+	+	-/+	-/-	-/+
Beef PLA/Nisin/Dill EO	+	-	+	-/+	-/-	-/-
Beef PHBV/Nisin/Dill EO	+	-	+	-/+	-/-	-/+

* - no CFU were identified + under 50 CFU ++ over 50 CFU +++ over 100 CFU

Challenge test

A "challenge test" was performed on the developed packaging materials using a bacterial strain of interest, namely *Escherichia coli* ATCC 8739. The test performed was of the "single challenge" type, namely a suspension of *Escherichia coli* ATCC 8739 (10^6 CFU) was added to the product in a proportion of 0.5 ml per 100 g of minced beef (Russel, 2003) and homogenized very well. There were prepared five samples for testing (Figure 3), as follows: - Control Sample – minced beef inoculated with the bacterial strain and stored at 4±0.5°C; - PLA/Nisin sample – minced beef inoculated with the bacterial strain and packed between 2 PLA/Nisin films, then stored at 4±0.5°C; - PHBV/Nisin sample – minced beef inoculated with the bacterial strain and packed between 2 PHBV/Nisin films, then stored at 4±0.5°C; - Nisin 1% sample - minced beef sample to which 1% nisin was added, inoculated with the bacterial strain and stored at 4±0.5°C; - Nisin 5% sample - minced beef sample to which 5% nisin was added, inoculated with the bacterial strain and stored at 4±0.5°C.

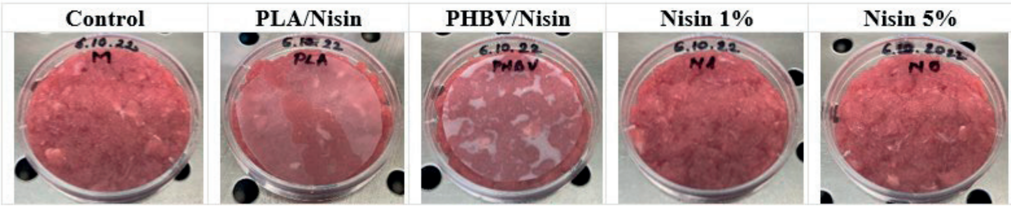

Figure 3. The appearance of the samples subjected to analysis

The samples were analysed after 24 and 48 hours to determine the growth rate of the applied bacterial strain. The results are further presented in Table 9. A decrease in the development of *E. coli* ATCC 8739 can be observed both after 24h and after 48h for all the samples in the presence of the tested films or the nisin, compared to the control sample, by approximately 1 logarithmic cycle. Applying nisin directly to minced beef was more effective, at the end of the storage period for these samples the lowest values were obtained. These results prove the antibacterial efficiency of nisin against *E. coli* ATCC 8739, on the studied product.

Table 9. Growth rate of *Escherichia coli* ATCC 8739 bacteria in the tested samples

Sample / Moment of analysis	0 h (lgCFU)	24 h (lgCFU)	48 h (lgCFU)
Control	5	4.30	4.95
PLA/Nisin/Dill EO	5	4.69	4.04
PHBV/Nisin/Dill EO	5	4.84	4.38
Nisin 1%	5	4.38	4.02
Nisin 5%	5	4.09	4.02

CONCLUSIONS

According to the obtained results, the minced beef samples packed in the presence of the two studied films (PLA/Nisin and PHBV/Nisin) and stored at 4°C demonstrated a good behaviour for 5 days, while the control sample started the process of degradation after only 3 days from packaging. The microbiological analyses showed that the microbial load of the tested samples had a continuous decrease during the refrigeration period for all the analysed samples. However, the samples packaged in the presence of PLA/Nisin and PHBV/Nisin films presented lower values of the microbial load, compared to the control sample, during the storage period, demonstrating that these materials have the potential to slow down the development of microorganisms in the tested minced beef samples.

Furthermore, nisin proved to be efficient against *E. coli* ATCC 8739, reducing the bacterial load in the tested samples with about 1 logarithmic cycle in 48 hours.

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