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PLA/PHBV ACTIVE PACKAGING APPLICATION ON FRESH MINCED CHICKEN MEAT

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Abstract

Meat spoilage is of great concern along the food chain, in respect to both consumers health and food waste, and also from an economic point of view. Spoilage mainly occurs due to the growth of microorganisms, from which the necessity of developing new packaging materials with antimicrobial properties have gained more and more interest. Active packaging materials can incorporate different ingredients that could interact with the food products and with the packaging environment in an active way. It presents great properties in respect to shelf-life prolongation, due to the variety of active substances that can be added (essential oils, natural antimicrobials, bacteriocins, etc.). The aim of the present study was to determine the effect that an active packaging material have on the properties and quality of fresh minced chicken meat. In this respect, a film based on PLA/PHBV and a nanoemulsion containing nisin and dill essential oil was applied on the studied meat, and its quality indicators (physical-chemical and microbiological parameters) were monitored during 7 days of storage at 4±1°C. The results showed that the packaging film maintained the chicken meat properties during the monitored storage period.

Key words*: fresh minced chicken meat, active packaging, PLA, PHBV, nisin, dill essential oil.*

INTRODUCTION

The production and consumption of broilers at a global level is increasing every year, with a prediction of production for 100 million tons and consumption of 98 million tons in 2022. Chicken meat is preferred by consumers due to its high nutritional value and unique taste and flavour (Shao et al., 2023). It is the most consumed meat in many countries due to its low-fat content, availability and relatively low cost. Fresh chicken meat is highly perishable due to high content in protein, free amino acids, vitamins and water (Mohamed-Noor et al., 2012; Wang et al., 2023), the main reason for spoilage being related to psychrotrophic microbial growth and physicochemical changes (Katiyo et al., 2020). The microbial contamination is primary generated from the bird's gastrointestinal track microbiota that can spread on the meat and equipment surface and also air within the processing area and during further processing steps through portioning and packaging (Hrustemović et al., 2022; Dourou et al., 2023). Furthermore, microbial spoilage can occur anytime during processing, retail and at the end consumer generating great economic losses besides health-related outbreaks. Due to this facts, low shelf life and increasing consumer demands for safer food products, research has been conducted to develop various technologies and/or packaging materials for chicken meat preservation.

Tsafrakidou et al. (2021) determined the evolution of spoilage microbiota of minced chicken meat during 10 days of storage at 4°C. The evaluation was made in association with pH changes and acetic and lactic acids in retail oxygen free modified atmosphere packaging (30 CO_2: 70 N_2). It was shown that the modified atmosphere packaging led to a growth inhibition of lactic acid bacteria (under 6.5 log CFU/g) and a total inhibition of *Enterobacteria*, *Pseudomonas*, *Enterococci*, *Staphylococci* and yeasts.

Karaca et al. (2023) developed real time CO_2 freshness indicators to be used on chicken breast, based on phenol red and bromothymol

blue. The developed indicators were applied to monitor chicken meat spoilage packed in polyamide/polyethylene pouches under air and 100% N_2 at 4°C for 10 days. The results of the study showed that the bromothymol blue based indicator was highly efficient in showing changes in meat quality, having three stage colour transition, namely dark blue-turquoise-green. The results shown by the freshness indicator were supported by the physicochemical, microbiological and sensorial properties of the tested samples.

Another area of research is related to the application of essential oils, which is the case of a study conducted by Chaichi et al. (2021), who tested the synergistic antibacterial effect of cinnamon, shirazi-thyme and clove essential oils both *in vitro* and *in vivo* in chicken breast meat against *Escherichia coli*, *Pseudomonas fluorescens* and *Staphylococcus aureus*. The most sensitive bacteria was proved to be *S. aureus*, while the most resistant to the essential oils action was *P. fluorescens*. The combination of the three essential oils presented a synergistic effect with a 6-8 folds reduction in the minimum inhibitory concentration compared to double combination or single use of the tested essential oils. The results were sustained by the *in vivo* evaluation of essential oils action, preventing the growth of *P. fluorescens* in chicken breast for 12 days. Takma and Korel (2019) developed an active film based on PET, chitosan and alginate coating, incorporated with black cumin oil. The developed material showed antimicrobial activity against *E. coli* and *S. aureus*, and modified quality indicators in chicken meat packed in the presence of the films were also observed. Another study, performed by Mulla et al. (2017), used clove essential oil as coating for LLDPE, which was previously treated at its surface with chromic acid. The results of the study showed that the material presented great antimicrobial activity when used as packaging for chicken meat, against *Listeria monocytogenes* and *Salmonella typhimurium*.

Another approach in this domain is the application of bacteriocins in food packaging. Guo et al. (2014) coated PLA films with mixtures of chitosan (in acid solution) and acetic, levulinic and lactic acids. Further, nisin and lauric arginate ester (LAE) were added to the solution alone or in combination. The developed films were applied on turkey meat. The results showed that the coatings containing chitosan and LAE reduced *Listeria innocua* with about 4.5 log CFU/cm^2, while nisin showed less efficiency than LAE. Zhou et al. (2015) studied two application methods of plantaricin BM-1 and its effect on *L. monocytogenes* and spoilage bacteria in sliced vacuum packed cooked ham. The application on the surface of the ham of plantaricin BM-1 was more effective than the incorporation of the bacteriocin in the product. However, regardless of the application method, plantaricin BM-1 inhibited the development of *L. monocytogenes* during the first 21 days of refrigerated storage. In another study, Barbiroli et al. (2017) developed an active packaging containing sakacin-A, which was further applied on thin-cut veal meat slices with the purpose of *Listeria* inhibition. The results of the study showed a 1.5 log reduction of *L. innocua* after 48 hours incubation at 4°C in the veal slices packed using the developed active packaging, compared to control samples.

Therefore, the aim of the present study was the evaluation of the application of an active packaging film based on PLA/PHBV and nanoemulsion containing nisin and dill essential oil on fresh minced chicken meat quality.

MATERIALS AND METHODS

To carry out this experiment, boneless skinless chicken breast purchased from a butcher shop in Bucharest was minced and then packed, forming the following samples:

(1) The film obtained by ES (PLA coated by electrospinning with nanoemulsion with dill essential oil and nisin) placed at the base of the PET casserole in direct contact with the tested sample - **Chicken PLA/Nisin/Dill EO**.

(2) The film obtained by ES (PHBV coated by electrospinning with nanoemulsion with dill essential oil and nisin) placed at the base of the PET casserole in direct contact with the tested sample - **Chicken PHBV/Nisin/Dill EO**.

(3) The PET casserole considered as a Control - **Chicken Control**.

In each casserole, 100 g of minced chicken meat was packed and stored at 4±0.5°C, to

determine the quality and shelf life during storage (Figure 1).

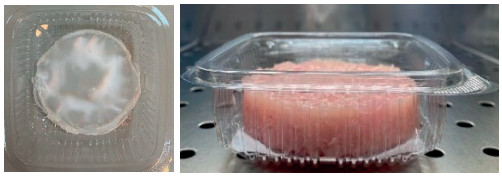

Figure 1. Experimental design

Further, free acidity determination was performed using the titration method with NaOH 0.1N in the presence of phenolphthalein as color indicator. pH was determined using a WTW INOLAB 720 series type pH meter, equipped with an automatic temperature compensator. Dry matter content (DM) was determined using a thermobalance type RADWAG MAC 50. A NOVASINA equipment was used for water activity (aw) determination. Freshness analysis consisting of the determination of free ammonia was evaluated using the Nessler reagent. Color determination was assessed with a HunterLab colorimeter, Miniscan XE Plus at room temperature. Furthermore, microbiological analysis were performed, consisting of TVC (total viable count), Enterobacteriaceae and *E. coli*/Coliforms determination, using dry medium plates (Compact Dry).

RESULTS AND DISCUSSIONS

The pH values (Table 1) had an increasing trend during the refrigerated storage period, for all the studied samples. However, values close to the initial pH value were obtained, the variations being insignificant. Further, the values obtained after determining the acidity of minced chicken meat samples showed a decreasing trend during the refrigerated storage period, the results being correlated with those obtained for the pH of the studied samples.

Table 1. Evolution of pH and free acidity of minced chicken meat during the refrigeration storage

Sample / Moment of analysis	pH				Acidity (oleic acid/100 g)			
	Day 0	Day 3	Day 5	Day 7	Day 0	Day 3	Day 5	Day 7
Chicken Control	6.07 ± 0.028	5.93 ± 0.000	6.42 ± 0.014	6.41 ± 0.183	6.805 ± 0.615	7.065 ± 0.190	5.93 ± 0.254	6.590 ± 0.735
Chicken PLA/Nisin/Dill EO	6.07 ± 0.028	5.99 ± 0.000	6.65 ± 0.063	6.66 ± 0.205	6.805 ± 0.615	7.445 ± 0.586	6.395 ± 0.360	6.230 ± 0.268
Chicken PHBV/Nisin/Dill EO	6.07 ± 0.028	5.94 ± 0.000	6.53 ± 0.000	6.62 ± 0.049	6.805 ± 0.615	6.915 ± 0.190	6.705 ± 0.289	6.705 ± 0.445

After analysing the data in Table 2, no significant changes were observed in the values obtained for dry matter for the analysed samples. As for aw, a trend of decreasing values can be observed for the chicken samples analysed and stored in a refrigerated state at a temperature of 4±0.5°C. 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 2. The values for dry matter (DM) and water activity (aw) of the tested samples during storage

Sample / Moment of analysis	DM%				a_w			
	Day 0	Day 3	Day 5	Day 7	Day 0	Day 3	Day 5	Day 7
Chicken Control	25.402 ± 2.013	25.615 ±0.327	25.991 ± 0.060	23.827 ± 0.779	0.988 ± 0.001	0.978 ± 0.002	0.972 ± 0.002	0.975 ± 0.000
Chicken PLA/Nisin/Dill EO	25.402 ± 2.013	25.365 ± 0.332	29.199 ± 4.570	25.140 ± 1.140	0.988 ± 0.001	0.978 ± 0.004	0.974 ± 0.000	0.972 ± 0.004
Chicken PHBV/Nisin/Dill EO	25.402 ± 2.013	27.611 ± 0.545	24.024 ± 0.837	25.349 ± 0.823	0.988 ± 0.001	0.973 ± 0.000	0.974 ± 0.000	0.971 ± 0.002

Regarding freshness analysis (Table 3), chicken samples packaged in the presence of films based on PLA and nisin and PHBV and nisin showed no signs of product degradation on day 7 (the last day of analysis), compared to the Control sample which showed signs of alteration on day 5 of analysis.

Table 3. NH3 presence in minced fresh chicken meat during refrigeration storage

Sample / Moment of analysis	Day 0	Day 3	Day 5	Day 7
Chicken Control	Negative	Negative	Weakly positive	Weakly positive
Chicken PLA/Nisin/Dill EO	Negative	Negative	Negative	Negative
Chicken PHBV/Nisin/Dill EO	Negative	Negative	Negative	Negative

Following the analysis of the colour of the minced chicken meat samples packaged in the presence of the studied films and stored in a refrigerated state at 4±0.5°C, it can be observed that the values of the parameters L*, a* and b* did not change significantly during the period of storage compared to the values obtained for the sample analysed on the day of packaging (Table 4), the samples maintaining their initial appearance during the storage period (Figure 2).

Table 4. The values of the L*, a* and b* parameters for the tested samples during refrigeration storage

Moment of analysis / Sample	Chicken Control			Chicken PLA/Nisin/Dill EO			Chicken PHBV/Nisin/Dill EO		
	L*	**a***	**b***	**L***	**a***	**b***	**L***	**a***	**b***
Day 0	56.71 ± 0.94	3.03 ± 0.40	14.57 ± 0.62	56.71 ± 0.94	3.03 ± 0.40	14.57 ± 0.62	56.71 ± 0.94	3.03 ± 0.40	14.57 ± 0.62
Day 3	56.33 ± 0.23	3.81 ± 0.24	15.70 ± 0.28	55.40 ± 0.40	4.87 ± 0.32	14.20 ± 0.14	55.41 ± 0.45	5.22 ± 0.25	15.91 ± 0.21
Day 5	57.64 ± 0.16	4.56 ± 0.18	15.26 ± 0.29	57.63 ± 0.21	4.43 ± 0.32	15.48 ± 0.27	55.40 ± 0.36	5.47 ± 0.16	16.13 ± 0.10
Day 7	56.94 ± 0.20	4.16 ± 0.36	14.57 ± 0.14	56.56 ± 0.25	4.56 ± 0.16	16.15 ± 0.20	55.58 ± 0.41	4.38 ± 0.09	16.75 ± 0.34

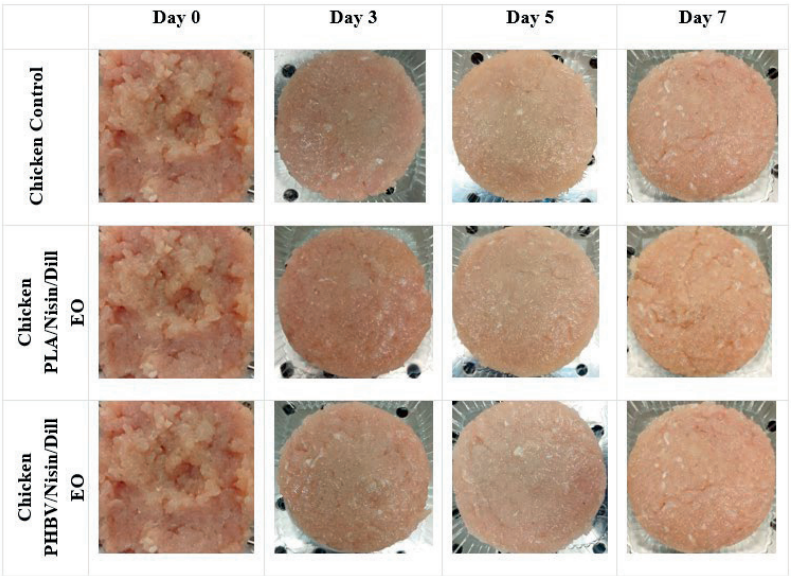

Figure 2. Appearance of minced chicken meat samples during the refrigerated storage period

Regarding the microbiological analysis, the evolution of NTG values, as shown in Table 5, shows an increase of approximately 1 logarithmic cycle during the storage period. However, at the end of the analysis period, the samples of minced chicken meat packaged in the presence of the tested films obtained lower values of this parameter compared to the control sample, which leads to the conclusion that the films used to package the product reduced the microbial activity within the samples.

Table 5. TVC values of fresh chicken meat during storage

Sample / Moment of analysis	Day 0	Day 3	Day 5	Day 7
	Total viable count (lgCFU)			
Chicken Control	4.060	4.91	5.21	5.57
Chicken PLA/Nisin/Dill EO	4.060	4.85	5.12	5.27
Chicken PHBV/Nisin/Dill EO	4.060	5.16	5.03	5.46

Table 6 shows both the presence of Enterobacteriaceae and *E. coli*/Coliforms in all analyzed samples, regardless of the packaging method. However, a microbial reduction can be observed in the samples packaged in the presence of films based on PLA/Nisin and PHBV/Nisin, compared to the control sample, during the refrigerated storage period at a temperature of 4±0.5°C.

Table 6. The values obtained following the determination of *E. coli*/Coliforms and Enterobacteriaceae

Sample / Moment of analysis	Enterobacteriaceae*				*E. coli*/Coliforms*			
	Day 0	Day 3	Day 5	Day 7	Day 0	Day 3	Day 5	Day 7
Chicken Control	+++	++	++	++	+/+++	+/+	+/+	+/+
Chicken PLA/Nisin/Dill EO	+++	++	+	+	+/+++	-/+	-/+	-/+
Chicken PHBV/Nisin/Dill EO	+++	++	+	+	+/+++	-/+	-/+	-/+

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

CONCLUSIONS

According to the results obtained, the minced chicken meat samples packed in the presence of the two studied films (PLA/Nisin/Dill EO and PHBV/Nisin/Dill EO) and stored at 4°C demonstrated a good behavior for 7 days, maintaining their quality, while the control sample started the process of degradation after only 5 days from packaging. The microbiological analyzes showed that the microbial load of the tested samples had a continuous decrease during the refrigeration period for all the analyzed samples. However, the samples packaged in the presence of PLA/Nisin/Dill EO and PHBV/Nisin/Dill EO 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 fresh minced chicken meat.

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