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RESEARCH ON EFFECT OF ACTIVE AND SUSTAINABLE ANTIMICROBIAL PACKAGING ON GROUND BEEF

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Abstract

Food packaging is innovating towards greener polymers and wider applications of bioactive compounds. Biodegradable active packaging based on natural compounds is a new approach, as it aims to improve shelf life. In this study, the active packaging materials used were chitosan/gelatin/clay polymer films in which sage, fennel, sea buckthorn nanoemulsions were incorporated. To carry out the experiments, the analysed meat was purchased from a chain of butchers in a store in Bucharest. For these experiments, chilled minced beef was chosen. Physical-chemical analyses were carried out on minced beef packed in chitosan/gelatin/clay polymeric films incorporated with nano-emulsions of sage, sea buckthorn, the control package being the polyethylene terephthalate casserole. The physical analyses performed during the experiments were: determination of free ammonia, water activity, colour analysis, analysis of dry matter and moisture content and determination of pH. The microbiological analyses consisted in determining the total plate count (NTC) and the presence of E. coli/coliform bacteria. Based on physical-chemical and microbiological analyses, both the freshness of the meat and its shelf life were determined, during seven days of storage (day 0, day 3, day 5 and day 7) at chilling temperature (4C). Following the results obtained in the physical-chemical analyses, we can state that there is a clear difference in the quality of the meat used in the experiments, during the storage period, depending on the packaging type used.

Key words*: food packaging, green polymer films, natural compounds, bioactive compounds, shelf life.*

INTRODUCTION

The accumulation of plastic waste in oceans and landfills has brought great threats to nature and human beings (MacLeod et al., 2021). Technological innovations in packaging are mainly related to the development of new polymeric materials and the combination of polymers with different properties (Espino-Pérez et al., 2016). It is necessary to develop sustainable packaging materials from biopolymers, which are renewable, degradable and compostable (Silva et al., 2020). Active packaging made of biodegradable polymers and natural additives is emerging as an ecological alternative to conventional packaging. Biodegradable active packaging based on natural compounds is a new approach, as it aims to improve shelf life and reduce food waste. Active packaging can be, for example, highlighted as having an antioxidant function capable of delaying or inhibiting the oxidation of packaged foods (Sharma et al., 2023).

Meat spoilage represents a great problem worldwide, affecting consumers health and leading to food waste, both having repercussions on economic aspects (Cercel et al., 2017; Popa et al., 2023). Research have been conducted in order to improve meat shelf life, minimize food waste and also minimise the impact that packaging have on the environment. Battisti et al. (2017) developed paper sheets coated with chitosan based solutions and packed beef meat. The packaging showed a lower microbial count in the packed beef samples compared to control, a greater stability in terms of lipid oxidation. New antibacterial packaging materials were developed by Lin et al. (2019), by incorporating chrysanthemum essential oil into chitosan nanofibers. The developed materials were tested on beef and showed an inhibition rate of *Listeria monocytogenes* of 99.91% at 4°C, 99.97% at 12°C and 99.95% at 25 °C, after 7 days of storage.

MATERIALS AND METHODS

Materials

To carry out the experiments, Figure 1, fresh minced beef was purchased from a chain of

butchers from a supermarket in Bucharest. Physical-chemical and microbiological analyses were carried out in triplicate during seven days of refrigerated storage at 4°C±0.5, namely on day 0 (initial analysis), day 3, day 5 and day 7 of storage. Therefore, for these experiments, 8 systems for minced beef packaging were prepared, as follows:

- Control sample, represented by commercial polyethylene terephthalate (PET) casserole - Control Sample;
- PET casserole + film of chitosan-gelatine-nano-clay-sage nanoemulsion - C/GE/Clay/Sage;
- PET casserole + chitosan-gelatine film- fennel nanoemulsion - C/GE/Clay/Fennel;
- PET casserole + chitosan-gelatine film- sage + sea buckthorn (SB) nanoemulsion - C/GE/Clay/Sage+SB;
- PET casserole + chitosan-gelatine film- fennel + sea buckthorn nanoemulsion - C/GE/Clay/Fennel+SB;
- PET casserole + Nanofiber electrospinning (ES) PLA - chitosan-gelatine-clay-sage nanoemulsion - ES PLA/C/GE/Clay/Sage;
- PET casserole + Nanofiber ES PLA- chitosan-gelatine-clay-sage nanoemulsion- ES PLA/C/GE/Clay/Fennel.

Figure 1. Aspect of packed beef using the developed packaging materials (own source)

Methods

During the refrigeration period, the following physical-chemical analyses were performed: determination of free ammonia (freshness analysis), water activity (a_w) index, colour analysis, moisture content and determination of pH. The microbiological analyses consisted of determining the total number of mesophilic aerobic germs and the presence of *E. coli*/ Coliform bacteria.

Briefly, free ammonia was determined using the Nessler reagent reaction method. 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. Colour determination was performed at room temperature using a HunterLab colorimeter, Miniscan XE Plus. Moisture content 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 (%). The pH determination was performed using a pH meter WTW INOLAB 720 series type with automatic temperature compensator. The microbiological analysis aimed at determining the total number of aerobic count and the presence of *E. coli*/Coliforms in the studied samples. For these analysis, specific Petri films with lyophilised culture media were used.

RESULTS AND DISCUSSIONS

Free ammonia

According to the results of the freshness analysis, the samples presented a negative Nessler reaction for 5 days, except the Control sample which presented a positive reaction. After 7 days of analysis, all samples presented positive Nessler reaction, meaning that their freshness started to degrade.

Water activity

Water has an important role to ensure food quality; its presence in food in certain amount provides both conditions for the development of spoilage microorganisms and enzymatic reactions that are directly responsible for the smell, colour and taste of food. The values of the water activity index (a_w) of the analysed samples during storage, are presented in Figure 2.

Water activity presented higher values for the Control sample with an increasing tendency over the storage period. For all samples packed in the developed packaging systems there can be observed that the values of a_w presented values lower compared to the Control sample, with a tendency of decreasing over the storage period. The lowest values were determined for samples ES PLA/C/GE/Clay/Sage and ES

PLA/C/GE/Clay/Fennel. 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.

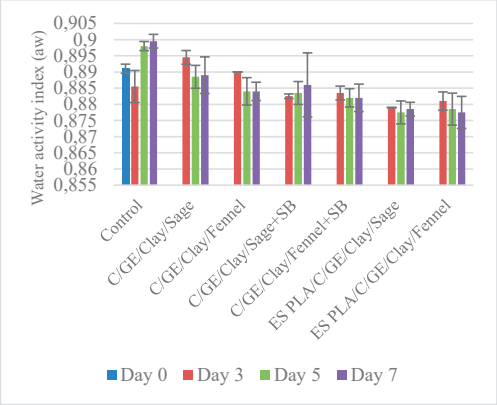

Figure 2. Water activity index (aw) values determined during the storage period at 4°C

Moisture content

The moisture content for the analysed samples is presented in Figure 3. On the last day of analysis, the Control sample registered a decreasing value of this parameter compared to the initial moment of analysis and compared to all analysed samples. During the storage period small variation were registered for the samples packed using the developed materials, however the obtained values were similar to the first day of analysis (Day 0).

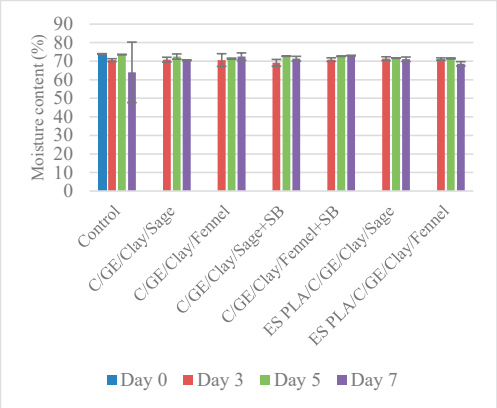

Figure 3. Values regarding the moisture content of minced beef during the storage period at 4°C

Determination of pH

The pH value indicates a number of characteristics of minced beef, such as colour, freshness, water holding capacity or juiciness. According to the results obtained (Figure 4), the meat samples packed in the active films and stored at a temperature of 4°C maintained until the seventh day pH values lower than 6, while the meat packed in PET casserole reached a pH of 6.21. For all samples these pH values are normal for beef stored at refrigeration temperature. It seems that the Control sample has an increased aging rate than samples packed in active films.

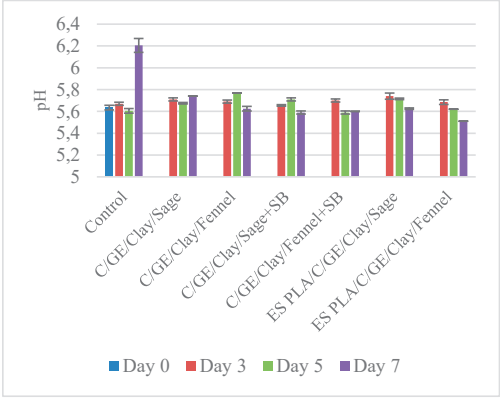

Figure 4. The evolution of the pH values of minced beef, during the storage period at the temperature of 4°C

Colour analysis

In the food industry, the colour of the products is an important attribute, because with its help the quality is appreciated. Colour measuring instruments are used to control the colour of ingredients and to evaluate the efficiency of processes in obtaining and maintaining the desired coloured food product. Colour measurements are also made for other purposes. These include: measuring raw and processed food quality indices for the use of data in quality control, documentation and communication; determining the conformity of food quality according to specifications; analysis of changes in the quality of food products as a result of food processing, storage and other factors. The results obtained following colour analysis are presented in Tables 1-3.

Table 1. Evolution of the L* parameter values for the studied samples

Sample / Moment of analysis	Day 0	Day 3	Day 5	Day 7
Control	33.079 ± 0.77	35.198 ± 0.37	31.119 ± 0.18	30.516 ± 0.21
C/GE/Clay/Sage	33.079 ± 0.77	30.610 ± 0.56	33.404 ± 0.27	30.815 ± 0.25
C/GE/Clay/Fennel	33.079 ± 0.77	29.160 ± 0.34	30.695 ± 0.12	33.138 ± 0.57
C/GE/Clay/Sage+SB	33.079 ± 0.77	29.817 ± 0.09	33.876 ± 0.36	32.599 ± 0.71
C/GE/Clay/Fennel+SB	33.079 ± 0.77	32.319 ± 0.31	29.453 ± 0.25	29.469 ± 0.32
ES PLA/C/GE/Clay/Sage	33.079 ± 0.77	31.302 ± 0.46	30.952 ± 0.38	41.423 ± 0.34
ES PLA/C/GE/Clay/Fennel	33.079 ± 0.77	30.457 ± 0.21	32.555 ± 0.16	30.701 ± 0.45

Table 2. Evolution of the a* parameter values for the studied samples

Sample / Moment of analysis	Day 0	Day 3	Day 5	Day 7
Control	17.123 ± 0.63	8.788 ± 0.18	11.597 ± 0,91	14.431 ± 0.34
C/GE/Clay/Sage	17.123 ± 0.63	9.657 ± 0.20	10.607 ± 0,31	12.821 ± 0.71
C/GE/Clay/Fennel	17.123 ± 0.63	11.715 ± 0.06	10.141 ± 0,58	7.643 ± 0.10
C/GE/Clay/Sage+SB	17.123 ± 0.63	10.193 ± 0.25	14.122 ± 0,20	9.620 ± 0.42
C/GE/Clay/Fennel+SB	17.123 ± 0,63	9.321 ± 0.20	11.986 ± 0,20	13.119 ± 0.51
ES PLA/C/GE/Clay/Sage	17.123 ± 0.63	10.950 ± 0.19	8.916 ± 0,64	7.257 ± 0.15
ES PLA/C/GE/Clay/Fennel	17.123 ± 0.63	19.550 ± 0.57	9.720 ± 0,42	12.319 ± 0.50

Table 3. Evolution of the b* parameter values for the studied samples

Sample / Moment of analysis	Day 0	Day 3	Day 5	Day 7
Control	16.750 ± 0.45	13.521 ± 0.10	14.257 ± 0.14	15.453 ± 0.13
C/GE/Clay/Sage	16.750 ± 0.45	13.440 ± 0.11	13.959 ± 0.14	13.920 ± 0.34
C/GE/Clay/Fennel	16.750 ± 0.45	14.235 ± 0.26	14.696 ± 0.17	12.925 ± 0.32
C/GE/Clay/Sage+SB	16.750 ± 0.45	14.102 ± 0.27	15.996 ± 0.20	14.062 ± 0.19
C/GE/Clay/Fennel+SB	16.750 ± 0.45	13.703 ± 0.13	14.307 ± 0.19	13.497 ± 0.20
ES PLA/C/GE/Clay/Sage	16.750 ± 0.45	14.088 ± 0.14	13.421 ± 0.15	15.155 ± 0.14
ES PLA/C/GE/Clay/Fennel	16.750 ± 0.45	16.785 ± 0.34	13.702 ± 0.19	13.703 ± 0.14

In general, the values of the parameters L*, a* and b* were slightly lower during the analysis period compared to the values obtained initially (Day 0). Therefore, the luminance of the samples (L*) showed lower values on days 3, 5 and 7 of the analysis, which shows that the samples had a slightly darker colour compared to the initial time of analysis. This is normal under the conditions of keeping the meat refrigerated, due to curing process. The values of the parameter a* decreased significantly compared to the initial moment of analysis for all the samples studied, a fact that shows the shift to a darker colour of the samples. A decrease in the values of this parameter was also observed in other studies and may be due to the oxidation of oxymyoglobin and metmyoglobin (Suo et al., 2016). The same trend was observed in the parameter b*, with lower values during the analysis period compared to the initial moment. Similar results were observed by Pereira Cardoso et al. (2016), who studied the effect of gelatine and chitosan-based coatings on beef colour. In this study, lower values of the L* parameter were also observed compared to the initial moment of analysis, this may be due to the coatings used.

Microbiological analyses

Microbiological analyses were performed to highlight the development of aerobic mesophilic bacteria, coliform bacteria and the presence of E. coli for the samples used in the experiments (Table 4). The results showed that in the samples that are stored in polymer films containing oils, the number of mesophilic aerobic germs is much lower, compared to the Control sample, which is stored in PET packaging. The ground beef subjected to the experiments was not contaminated with E. coli. In none of the 7 days of analysis were found E. coli colonies, neither in the Control sample nor in the samples packed in active films. After the first 24 hours of storage, colonies of coliform bacteria were found in the minced beef from the Control sample, however coliforms were further

determined only in the Control sample during storage, while the active packaging proved to be efficient in the inactivation of this type of bacteria during storage.

Table 4. Microbiological analysis results - total number of aerobic mesophilic germs in ground beef samples stored at 4ºC

Analysed parameter / Storage time (days)	Total Plate Count (log CFU)				*E. coli* / Coliforms (log CFU)			
	0	3	5	7	0	3	5	7
Control	2.92	3.72	4.36	5.4	- / 2.04	- / 3.36	- / 3.61	- / 3.98
C/GE/Clay/Sage	2.92	3.04	3.93	4.98	- / 2.04	- / -	- / -	- / -
C/GE/Clay/Fennel	2.92	3.56	2.08	4.14	- / 2.04	- / -	- / -	- / -
C/GE/Clay/Sage+SB	2.92	3.27	4.11	4.34	- / 2.04	- / -	- / -	- / -
C/GE/Clay/Fennel+SB	2.92	3.72	4.12	5.32	- / 2.04	- / -	- / -	- / -
ES PLA/C/GE/Clay/Sage	2.92	3.84	4.29	5.07	- / 2.04	- / -	- / -	- / -
ES PLA/C/GE/Clay/Fennel	2.92	3.41	4.08	4.61	- / 2.04	- / -	- / -	- / -

- absence of microorganism

CONCLUSIONS

According to the obtained results, the minced beef samples packed in the presence of the six studied films (C/GE/Clay/Sage; C/GE/Clay/Fennel; C/GE/Clay/Sage+SB; C/GE/Clay/Fennel+SB; ES PLA/C/GE/Clay/Sage; ES PLA/C/GE/Clay/Fennel) 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 decreas during the refrigeration period for all the analysed samples compared to the control sample. Therefore, the developed packaging materials could be used for meat packaging, showing good properties in terms of shelf life prolongation.

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