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Influence of Active Packaging Covered with Coatings Containing Mixtures of *Glycyrrhiza* L. and *Scutellaria baicalensis* Extracts on the Microbial Purity and Texture of Sliced Chicken Sausages

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Abstract: Sliced chicken sausages were packed into polyethylene (PE) bags (control samples) and PE bags were covered with active coatings. The sausage slices were separated into two categories: non-coated (control samples) spacers and spacers covered with the antimicrobial coating. The chicken sausage slices were stored at 5 °C and examined after 72 h and 144 h storage times. Results obtained in this work demonstrated that the springiness of the chicken sausage slices decreased after 72 h of storage for all of the analysed packaging bags/films. Different results were obtained after 144 h of storage. In contrast to the samples stored in uncoated bags, the springiness of sausage slices stored in the active packaging decreased. Textural parameters with regards to chewiness, gumminess and cohesiveness were found to be greater after 72 h of storage for samples stored in the uncoated bags than for the sausage stored in active packaging materials. Contradictory results were observed after 144 h of storage. It was found that water loss from the sliced chicken sausage in active bags was lower than in uncoated PE bags. Microbial analysis showed that the packaging material covered with a coating containing a mixture of *Scutellaria baicalensis** and *Glycyrrhiza* L. extracts in the ratio of 1:2 was found to be more effective against mesophilic bacterial cells than a coating containing the mixture of these extracts in the ratio of 2*:1 after 72 h. The effect of active coatings on the number of bacterial cells was negligible after 144 h of storage.

Keywords: chicken sausage; *Scutellaria baicalensis* extract; *Glycyrrhiza* L. extract; antimicrobial coatings/packaging; sausage texture

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1. Introduction

The poultry sector has become one of the fastest-growing sectors within the livestock economy, as this meat is a very popular food commodity due to its high nutritional value, distinct flavour, low fat content and low production cost. As a result, the consumption of processed chicken products has significantly increased over the last twenty years, including emulsion-based sausages, chicken sausages, patties, meatballs and nuggets. This is the reason why an extension of shelf-life in chicken products has become a major concern for the poultry industry [1]. Sausages, including chicken sausages, are important, ready-to-eat meat products that are suitable for a healthy, more modern lifestyle [2]. The manufacturing of chicken sausage involves a number of handling steps, making them highly susceptible to contamination by microorganisms responsible for chicken meat spoilage that can be pathogenic for humans. The nutrient richness and high water content of sausage can enhance microbial growth. Bacteria, such as *Staphylococcus* spp., *Listeria monocytogenes*, *Salmonella* spp., etc., are common causes of foodborne infections associated with chicken sausage [3–8]. It should be noted that contamination during handling and cutting is more

effective for reducing the shelf-life/storage time of this type of sliced sausage than the initial contamination of raw food product (meat) [2]. Microorganism growth leads to the release of off-odours and off-flavours, as well as discolouration and slime production, thereby leading to decreased shelf-life and food quality. To overcome this problem, food manufacturers have used antimicrobial substances as food preservatives, such as lactate, nisin, acetate, potassium sorbate or sodium benzoate, which not only prevent undesirable growth but also extend the shelf-life of meat products [1,2]. In order to improve the quality of ready-to-eat meat products, several methods, e.g., pressure and heat processes, electron beam radiation and gamma radiation, can also be used to reduce the number of bacterial cells. Unfortunately, some of these physical and mechanical processes cause changes in the structure and texture of food products and even the possibility of chemical substances remaining on meat surfaces, which may lead to health problems in humans [2–5].

However, the use of active coating packaging for the preservation of sliced chicken sausages may decrease the number of microorganisms responsible for meat spoilage and increase product shelf-life. Hence, active packaging could become an important inclusion to maintain or even prolong the storage time of meat products such as sliced chicken sausages, while ensuring their quality, safety and integrity [1,2,6–10]. The fabrication of one layer, bi-layer, tri-layer or even multilayer films through the use of a surface-covering technology uses many biobased carriers and antimicrobial compounds in order to obtain active layers, which may lead to increased food shelf-life [10]. Plant extracts contain a wide range of bioactive components that include iridoids, polyphenols, saponins, amides, glycosides and alkaloids, as well as terpenoids, tannins and quinones. There are three main antimicrobial action mechanisms offered by plant extracts, which inhibit (a) cytoplasmic membrane function; (b) nucleic acid synthesis and (c) ATP synthesis. Additionally, active compounds extracted from plants can influence biofilm formation by affecting the quorum sensing mechanism [10–13].

Glycyrrhiza glabra L. is described as having many health-promoting properties, such as antimicrobial activity [14]. Glycyrrhizin is a major component of plant extracts which were also observed to contain carbohydrates, proteins, lipids and even essential oils [15,16]. It was shown that the extracts of *G. glabra* contain flavonoids with C5 aliphatic residues, which were noted to be constituents of licorice and are active against methicillin-resistant *Staphylococcus aureus* (MRSA). They were also proven to be effective against Gram-negative bacteria, e.g., *Klebsiella pneumoniae* and *Escherichia coli*, or Gram-positive, spore-forming strains, such as *Bacillus subtilis* [17]. In addition, extracts of *Scutellaria baicalensis* contain a wide variety of polyphenols, especially flavonoids. The most important of these compounds are baicalin, baicalein, wogonin, wogonoside and norwogonin. They also contain active essential oils, glycosides, lignan sterols and amino acids. It was demonstrated that these extracts exhibit significant antimicrobial effectiveness against Gram-negative and Gram-positive microorganisms [18–21].

Based on the results of a previous study [22], it was assumed that *S. baicalensis* and *Glycyrrhiza* L. extracts did not inhibit Gram-negative bacteria growth. They only decreased the number of *E. coli* cells. Opposite results were noted in the case of the extract mixtures. The results demonstrated the *Glycyrrhiza* L. and *S. baicalensis** extracts mixtures (m1—1*:2; m2—2*:1; m3—1*:1) inhibited the growth of *B. subtilis*, *S. aureus*, *E. coli* and *Pseudomonas syringae* strains. As clearly shown in this study, a synergistic effect between the two extracts was noted. Consequently, coatings containing 25% of the mixtures of extracts were prepared (M1—coating containing m1, M2—coating containing m2 and M3—coating containing m3). The results of this study showed that the active layers completely inhibited the growth of *B. subtilis* strain. In the case of the *S. aureus* cells, a 3-log decrease in the number of the viable cells was observed. It was demonstrated that all of the described coatings (M1, M2, M3), which contained mixtures m1, m2 and m3 as antimicrobial additives, did not inhibit the growth of *E. coli* strain and did not decrease the number of these bacteria cells. Similarly, the active layers were proven to not be active against the *P. syringae* strain. Therefore, it

was assumed that, in order to increase the activity of the coatings, the concentration of the extract mixtures in the coating-forming carrier should be increased up to 50%.

The goal of this work was to investigate the influence of packaging covered with active coatings containing *S. baicalensis** and *Glycyrrhiza* L. extracts as active agents on microbial purity and texture of the sliced chicken sausage.

2. Materials and Methods

Fresh chicken sausages were purchased from a local butcher's shop and brought (in PE bags) to the Center of Bioimmobilisation and Innovative Packaging Materials (CBIMO).

Polyethylene foil (A4, 50 µm) (KB FOLIE, Warsaw, Poland) was used as packaging material. Methylhydroxypropyl cellulose (MHPC) (Chempur, Piekary Śląskie, Poland) was used as a coating carrier in the tests. Decyl glucoside and caprylyl/capryl glucoside (Greenaction Sp. z o.o., Kielce, Poland) were used as emulsifiers. The emulsifiers were used to increase the adhesion of coatings to PE foil. *Glycyrrhiza* L., (Planteon, Borków Stary, Poland) and *S. baicalensis* (MyVita, Białystok, Poland) were used to extract active compounds. The ethanol (EUROCHEM BGD Sp. z o.o. Tarnów, Poland) was used as solvent to prepare plant extracts. To verify the coliform bacteria count of samples, Violet Red Bile Glucose Agar (VRBG) was used (Merck KGAA, Darmstadt, Germany). To verify the total bacterial count and the *S. aureus* count of the sliced chicken sausage, PPS (PPS: 0.85% m/v NaCl, 0.1% m/v peptone), PCA (BTL, Łódź, Poland) and Baird-Parker media (Merck KGAA, Darmstadt, Germany) were used. To determine *Salmonella* sp. count, Muller–Kauffmann Tetrathionate-Novobiocin (MKTTn), Rappaport Vassiliadis Broth, BGA and XLD (Scharlau, Barcelona, Spain) media were applied. To determine *L. monocytogenes* count, Half Fraser broth, Fraser broth and Fraser agar were all prepared. The media were made according to BTL, Merck and Scharlau protocols. The media were prepared according to the manufacturer's instructions. All media, except XLD and VRBG agars, were weighed, then suspended in 1 L of distilled water and sterilised at 121 °C for 15 min. XLD and VRBG agars were weighed, then suspended in 1 L of distilled water and heated to boiling.

2.1. Coatings Preparation

Initially, *S. baicalensis* and *Glycyrrhiza* L. extracts were prepared according to the method described in the previous study [20]. As a next step, two 50% systems of coating carrier containing the mixtures of *S. baicalensis** and *Glycyrrhiza* L. extracts (in m1—1*:2 and m2—2*:1 ratio) were prepared. The plant extracts were mixed at a weight ratio of 1:2 (m1) and 2:1 (m2) in beakers using a magnetic stirrer (500 rpm, Ika, Warsaw, Poland). Then, 2 g of caprylyl/capryl glucoside and 2 g of decyl glucoside were introduced into each extract solution and mixed for 5 minutes using a magnetic stirrer at 1000 rpm (Ika, Warsaw, Poland). The emulsifiers were added to increase the adhesion of coatings to PE foil. Simultaneously, 16 g of MHPC was introduced into 184 mL of water. The solution was mixed for 2 h using a magnetic stirrer (Ika, Warsaw, Poland) at 1000 rpm. An amount of 50 g of 8% MHPC solution was then introduced into 50 g of each mixture (m1 and m2) containing emulsifiers. The M1 (m1 + MHPC with emulsifiers) and the M2 (m2 + MHPC with emulsifiers) were mixed separately for 15 minutes using a magnetic stirrer (Ika, Warsaw, Poland) at 1000 rpm.

A polyethylene (PE) film was covered with M1 and M2 coatings using Unicoater 409 (Erichsen, Hemer, Germany) at a temperature of 25 °C with a 40 µm diameter roller. The coatings were dried for 10 min at 50 °C. A total of 1.6 g layers of MHPC, containing mixtures of extracts per 1 m^2 of polyethylene foil, was obtained. PE film was coated from one side to obtain bags and from both sides to obtain spacers. The PE film that was not covered with any coating was used as the control sample (C). Covered and non-covered films were cut and used to prepare bags and to obtain square foil spacers. To obtain the bags, covered and non-covered films were joined using a welder (HSE-3, RDM Test Equipment, Hertfordshire, Great Britain) in normal air conditions. The welding parameters were temperature—117 °C, pressure—4 kN, time—4 s.

2.2. SEM Analysis

The PE foil and PE covered with the active coatings M1 and M2 were analysed using a scanning electron microscope (SEM). A microscopic analysis was performed using a Vega 3 LMU microscope (Tescan, Brno-Kohoutovice, Czech Republic). The tests were important to determine if the PE foils were thoroughly and homogenously covered with active coatings. An analysis was carried out at 25 °C with a tungsten filament and an accelerating voltage of 10 kV was used to capture SEM images for both the non-covered and covered PE foil samples. All specimens were viewed from above.

2.3. Packaging and Storage

Chicken sausages were cut into 10 mm slices (Figure 1a); the portions were then packed into polyethylene bags and were welded. The sausage slices were aseptically introduced into bags. Each chicken sausage slice was separated with a square spacer (Figure 1b) and introduced into a bag (Figure 1c):

a. PE bags (control samples), slices separated with PE spacers;
b. PE bags covered with M1 coating, slices separated with PE spacers which were covered with M1 coating on both sides;
c. PE bags covered with M2 coating, slices separated with PE spacers covered with M2 coating on both sides.

(**a**) (**b**) (**c**)

Figure 1. The sliced chicken sausage (**a**) "0" samples; (**b**) "0" samples separated with spacers; (**c**) the samples inside the package before storage.

The chicken sausage slices were in contact with the active coatings on both sides.

Next, the bags were joined using a welder (HSE-3, RDM Test Equipment, Hertfordshire, Great Britain) in normal air conditions. The welding parameters were temperature—117 °C, pressure—4 kN, time—4 s.

The bags containing chicken sausages were then stored in a refrigerator at 5 °C. The samples were examined after 72 h and 144 h of storage.

2.4. Textural Analysis

The textural analysis of the chicken sausage (10 mm slice) was performed according to the PN-ISO 11036:1999 standard: "Sensory analysis. Methodology. Texture profiling" [23]. The tests were carried out using Zwick/Roell Z 2.5 (Wrocław, Poland).

2.5. Microbiological Analysis

For microbiological analysis, 10 ± 0.1 g of individual chicken sausage slices was aseptically introduced into a sterile stomacher bag along with physiological saline peptone solution (PPS: 0.1% m/v peptone; 0.85% m/v NaCl). The chicken sausage slices were homogenised in a Bag Mixer (Interscience, Saint-Nom-la-Brèteche, France) for 60 s and appropriate decimal dilutions were prepared in PPS. The total count was analysed according to PN-EN ISO 4833-2:2013-12 [24], the *S. aureus* count was determined according to PN-EN ISO 6888-1 [25] and the total coliform bacteria count was determined according to PN-ISO 4832:2007 [26]. To determine *Salmonella* sp. count, 25 ± 0.1 g of an individual chicken sausage slice was aseptically introduced into 225 mL of sterile PPS. The total *Salmonella* sp.

count was determined after 18 h of incubation according to PN-EN ISO 6579-1:2017-04 [27]. To determine *L. monocytogenes* count, 25 ± 0.1 g of an individual chicken sausage slice was aseptically introduced into 225 mL of sterile Half Fraser broth. The total *L. monocytogenes* count was determined according to PN EN ISO 11290-1:2017 standard [28].

2.6. Dry Mass Tests

Dry mass was analysed for fresh chicken sausage before being added into bags and after 72 h and 144 h of storage. Dry mass test was performed using a Weight Dryer (Radwag, Warsaw, Poland). The test was carried out in duplicate.

2.7. L a* b* Tests*

Chicken sausages' colour was investigated as an average of 9 measurements from randomly selected sausage slice spots with colorimeter (NR 20 XE, EnviSense) and related data software. Colour was measured through an aperture (diameter 8 mm) using the CIE L* a* b*colour space with a standard 10 observer and Illuminant D65. The selected parameters (to describe the results) were ΔE_{lab} (total colour aberration) and ΔL (the difference between lightness and darkness). The parameters were calculated according to EnviSense protocol.

2.8. Statistical Analysis

Statistical significance was determined using an analysis of variance (ANOVA) followed by a one-way ANOVA test. The values were considered as significantly different when $p < 0.05$. All analyses were performed with GraphPad Prism 8 (GraphPad Software, Version 9, San Diego, CA, USA).

3. Results

3.1. SEM Analysis

SEM analysis demonstrated that the polyethylene used in the experiments was slightly matte. Matte PE foil might have been obtained during film formation if a special calender with modified surface was used. SEM micrographs showed slightly rough surface of the uncoated film (Figure 2: SEM images under magnification of 200× and 1000× and in Supplementary Materials SEM images under 500× and 2000× magnifications). As shown in Figures 3 and 4, active layers (M1 and M2) did not affect the PE surface morphology. Moreover, it can be noticed that the PE film was thoroughly and homogenously covered with the coatings (coated films micrographs reflected uncoated films). The homogenous covering of PE film with the M1 and M2 layers may affect release of active substances from the entire surface of the coating to the food product. Moreover, as seen in Figure 4, small cracks were visible on the M2 coating which could have caused the faster release of active compounds from M2 coating. Comparing coatings M1 and M2 led to the observation that the M1 coating could have been active against bacteria longer than M2 coating.

3.2. Microbial Analysis

Results of the work showed that the number of mesophilic bacterial cells from the sliced chicken sausage stored in bags without of active coatings (C—control sample) increased after 72 h and 144 h of storage at 5 °C in air conditions (compared to the "0" sample—before storage). Figure 5 demonstrates that an M1 coating containing 50% of the mixtures of *Glycyrrhiza* L. and *S. baicalensis* extracts had an influence on the growth of microorganisms. It inhibited the growth of microorganisms after 72 h of storage. A lower than 1-log decrease in the number of viable cells was noted (compared to the "0" sample—before storage) for the samples stored for 72 h in bags covered with the M2 coating. It is tempting to suggest that the coatings were effective against mesophilic bacteria and that M1 coating was significantly more active than M2 active layer. As emphasised below (Figure 5), the PE films coated with M1 layer had an insignificant influence on the total count and the PE bags covered with the M2 coatings had no influence on the number of mesophilic bacteria compared to the bags without coatings after 144 h of sliced chicken

sausage storage. It was noted that the number of bacteria for the sausage samples stored in PE bags and in PE bags that were covered with the active coatings was higher (when compared to the "0" sample—before storage) after 144 h of storage. Statistical analysis showed that the differences between the numbers of bacteria were not significant ($p > 0.05$).

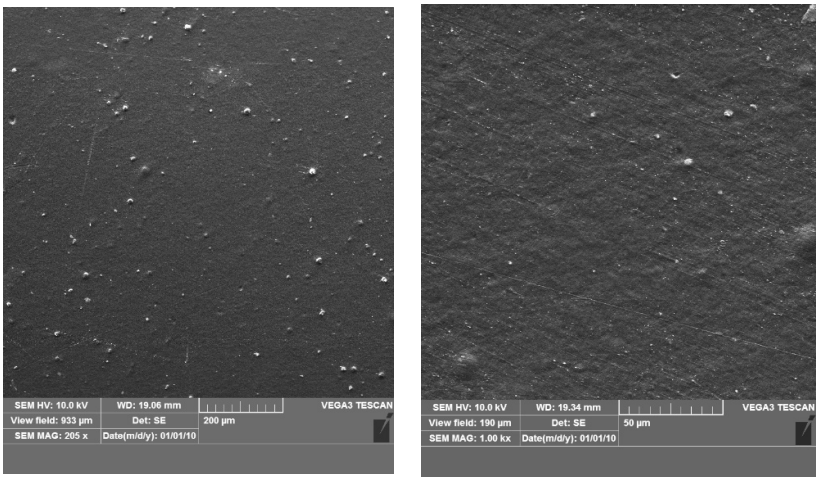

Figure 2. The PE film.

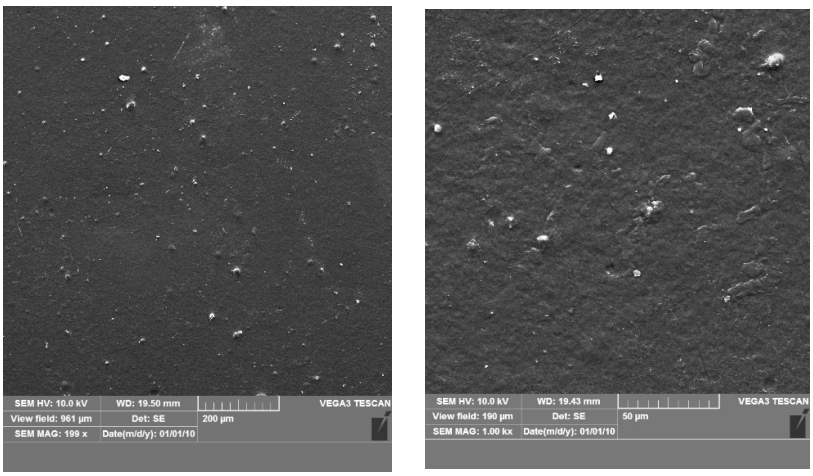

Figure 3. The PE film-covered M1 coating.

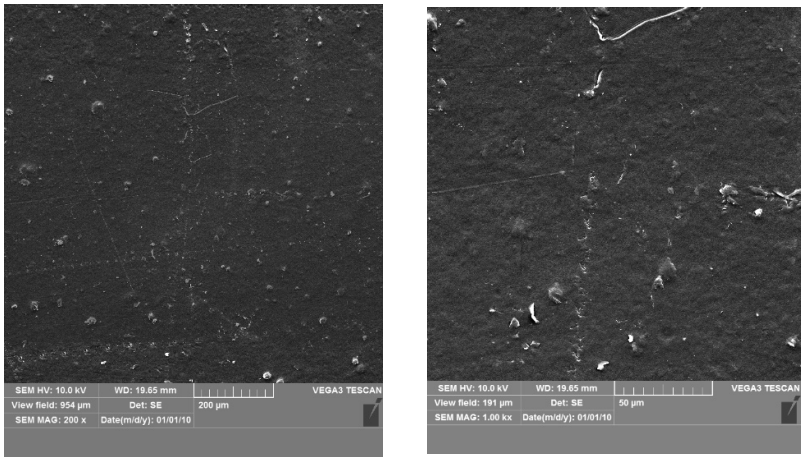

Figure 4. The PE film-covered M2 coating.

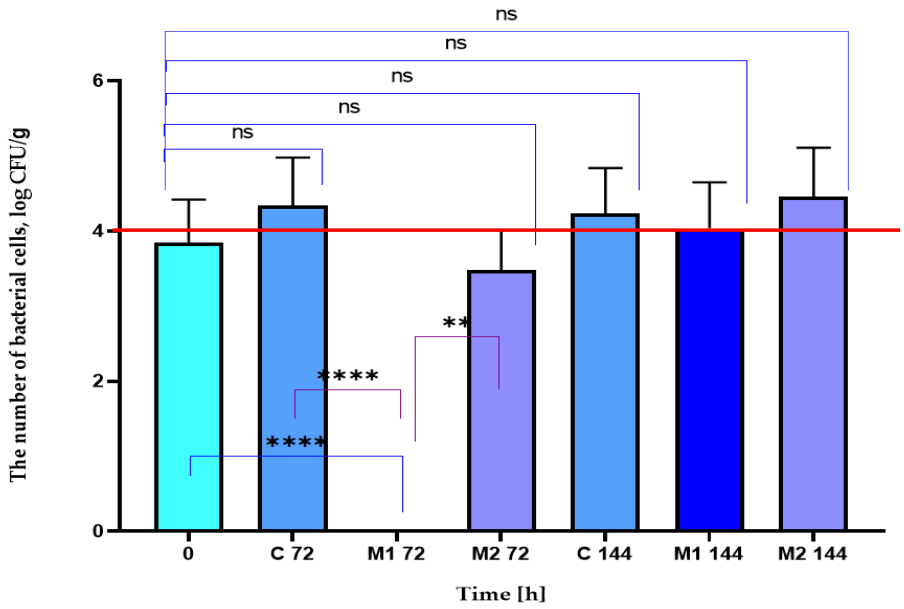

Figure 5. The total count of mesophilic bacteria. ANOVA: ns—not significant; ****—$p < 0.0001$; **—$p < 0.01$.

From a microbiological point of view, the acceptable limit for total viable count of chicken meat products is 6–7-log CFU/g [29]. However, it should be mentioned that, according to Grzybowski et.al. [30], a total count lower than 10^3 CFU/g is an acceptable/satisfactory number of living cells for sliced ready-to-eat meat products [30]. According to the author [30], the 4-log CFU/g is generally considered a maximum acceptable threshold of microbial load for sliced meat products. A higher number than 10^4 CFU/g is seen as unacceptable microbial load for sliced sausage to be consumed. This means that in contrast to the samples stored in PE bags without active coating or in active bags covered with the M2 active layer, the storage time of the sliced chicken sausage stored in bags coated with M1 coating may be 144 h, as the number of mesophilic bacteria for these meat products was still seen as an acceptable microbial load for the sliced sausage to be consumed. A previous work [22] proved that MHPC layers containing 25% of the *Glycyrrhiza* L. and *S. baicalensis** extracts (in m1—1*:2 and m2—2*:1 ratio), as active agents inhibited the growth of *B. subtilis* and decreased the number of *S. aureus* but did not have an influence on the number of Gram-negative bacteria. The results of that work proved that MHPC coating with a higher (50%) concentration of m1 mixture (M1) was active against mesophilic bacterial cells for the samples that were stored for 72 h. This coating only slightly decreased the number of living cells for sausage samples after 144 h of storage. The results of this work have also indicated that an MHPC coating with 50% of m2 mixture (M2) was slightly effective against mesophilic bacteria for the sausage slices that were stored for 72 h and was not effective against the bacteria for the samples that were stored 144 h. The low antibacterial activity of M2 layer might have been caused by faster release of antimicrobial agents from the coating and this was confirmed by SEM analysis (cracks visible on SEM micrographs).

The number of mesophilic bacterial cells from sliced chicken sausage for the control sample ("0" sample—before storage) was 3.85-log CFU/g (almost 4-log CFU/g), meaning that the sausage must have become contaminated during slicing.

According to Grzybowski et al. [30], the number of coliform bacteria in 10 mg of the sliced meat products should be 0. The *L. monocytogenes* cells should not be present in 1 g of the samples and *Salmonella* sp. should not be present in 25 g of sliced sausage. The number of the *S. aureus* cells in 1 g of the sliced sausage may be 100 for this product to be acceptable for consumption. Results from this study showed that no coliform bacteria (*L. monocytogenes*, *S. aureus* nor *Salmonella* sp.) cells were detected in the "0" sample nor

in any of the analysed sliced chicken sausage stored in uncoated PE bags and PE bags covered with the active coatings. It was seen that all analysed samples were acceptable for consumption even after 144 h of storage.

3.3. Textural Analysis

The results of the work showed that the springiness of the sliced chicken sausage decreased after 72 h of storage in uncoated PE films. After 144 h, this parameter increased. A modification of the bags with an active coating containing the m1 mixture as an active agent caused a greater decrease in springiness of the sliced chicken sausage after 72 h of storage compared to the uncoated bags. It was noted that after 144 h of storage, the chicken sausage samples in bags coated with the M1 layer caused a lower increase in springiness than when compared to the uncovered bags (Figure 6). Analysing the samples which were coated with the M2 layer, it was seen that springiness decreased after 72 h of storage. The greatest decrease in springiness was noted after 144 h of storage. The differences between springiness values were noticed to not be significant and this was confirmed by statistical analysis ($p > 0.05$).

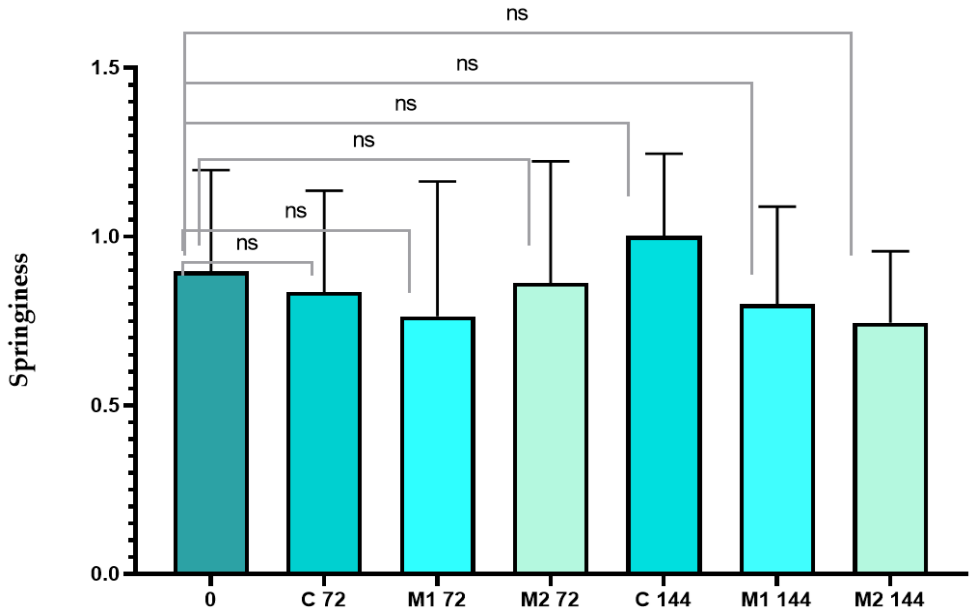

Figure 6. The springiness of the sliced chicken sausage. ANOVA: ns—not significant.

As emphasised below (Figure 7), the gumminess of sliced chicken sausage stored in bags without active coatings significantly increased after 72. After 144 h, the gumminess also increased when compared to the "0" sample. However, the increase was lower than when compared to samples stored for 72 h. These changes were not significant, as corroborated by statistical analysis ($p > 0.05$). When analysing the samples which were stored in packaging covered with the active coatings (M1 and M2), it was noted that gumminess increased after 72 h of storage. After 144 h of storage, the average gumminess of the sliced chicken sausage stored in active packaging increased when compared to that of the slices stored for 72 h and to the "0" sample. The differences between the samples were noticed to be significant, again verified by statistical analysis ($p < 0.01$). Comparing the samples after 72 h of storage in uncoated PE bags with the samples stored in coated bags, the gumminess values of the sliced chicken sausage was lower for the samples stored in active packaging than that of the of the samples stored in the uncoated bags. Contrary results were obtained for 144 h of storage. The active packaging caused a greater increase in gumminess than the uncoated PE bags. The differences between the gumminess values of the samples stored for 144 h were significant, verified by statistical analysis.

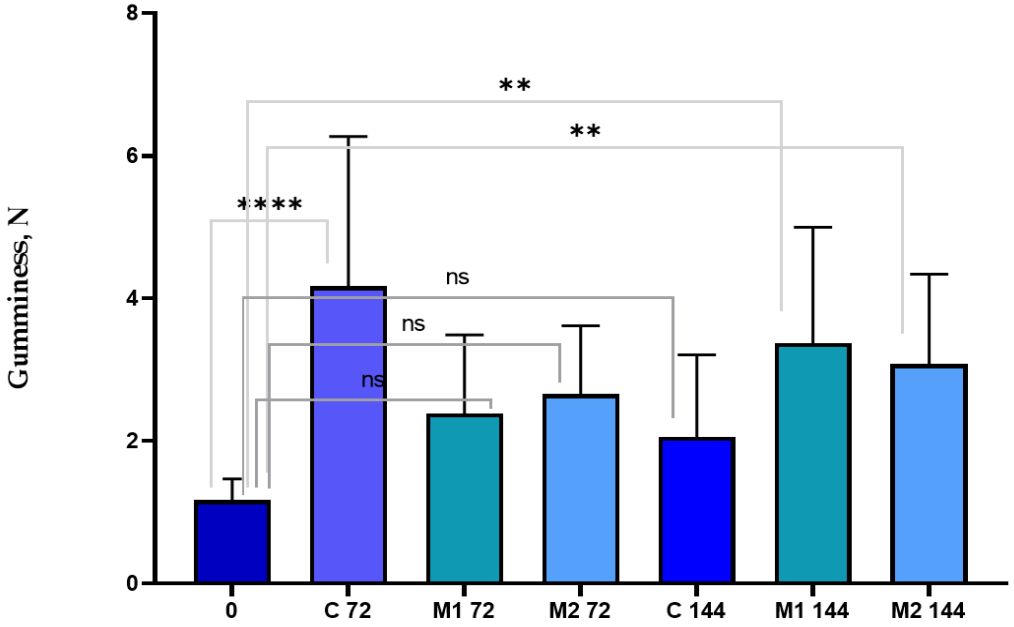

Figure 7. The gumminess of the sliced chicken sausage. ANOVA: ns—not significant; ****—$p < 0.0001$; **—$p < 0.01$.

In the case of another texture parameter, it was noted that the average cohesiveness value for the sliced chicken sausage stored in PE bags used as control samples increased after 72 h of storage (Figure 8). The significance of these changes was corroborated by statistical analysis ($p < 0.001$). The cohesiveness value, analysed for the same samples, when stored for a longer time (144 h) also increased, though the change was not significant. An increase in this parameter was also noted for slices stored for 72 h in bags covered with M1 and M2 coatings. After 144 h storage, an increase in sausage cohesiveness was determined. In addition, the differences in parameter values were higher than the cohesiveness values observed after 72 h, these changes were found to be significant.

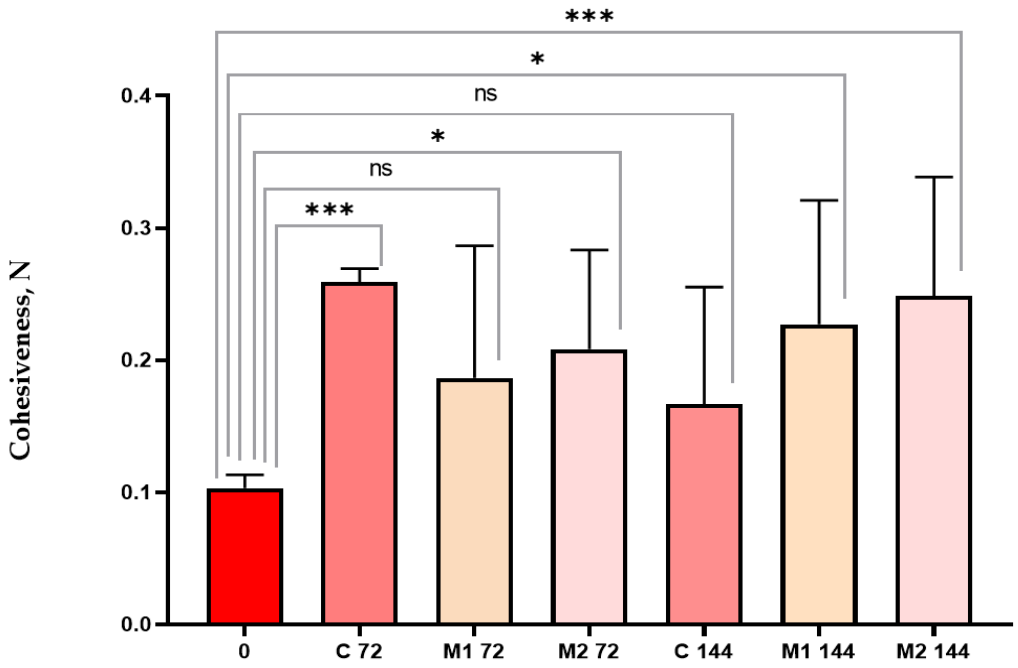

Figure 8. The cohesiveness of the sliced chicken sausage. ANOVA: ns—not significant; ***—$p < 0.001$; *—$p < 0.05$.

Analysing the increase in the cohesiveness and gumminess values after short-term storage (72 h), it was shown that an increase in these two parameters was lower for the sausage slices stored in active bags containing active spacers than for those stored in uncoated packaging.

The results also showed that chewiness depended on the packaging material after 72 h of storage (Figure 9). Investigating the chewiness values of the sliced chicken sausage that were stored in bags without active layers or coated PE films (M1 and M2) for 72 h, it was observed that this parameter increased. Additionally, the chewiness values of the samples stored in uncoated films significantly increased ($p < 0.0001$). The increase in the samples stored in active coatings was lower. After 144 h storage time, an increase of the chewiness was noted to be significant and this was corroborated by statistical analysis, but only in the case of samples stored in active packaging. The chewiness of sausage samples that were introduced into the bags that were uncovered increased insignificantly after 144 h storage. Results demonstrated that the modification of PE films with M1 and M2 active coatings caused lower changes in chewiness values than uncoated films, though only for 72 h of storage. As emphasised below (Figure 9), the increase of chewiness was lower for the M1 coating than for M2 layer.

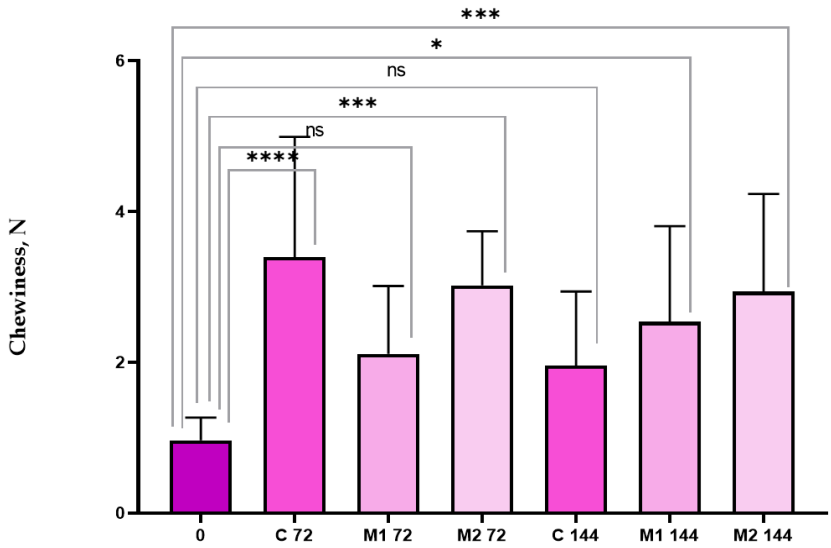

Figure 9. The chewiness of the sliced chicken sausage. ANOVA: ns—not significant; ****—$p < 0.0001$; ***—$p < 0.001$; *—$p < 0.05$.

3.4. Dry Mass Analysis

The results of this work showed that the dry mass of sliced chicken sausage was 66.8%. The storage of sausage in PE films led to an increase in this parameter to 68.75% after 72 h storage and to 69.40% after 144 h. It was observed that the dry mass of sliced chicken sausage stored in bags coated with M1 layer was higher than the dry mass of slices stored in bags without antimicrobial coating after 72 h and lower after 144 h of storage. The M2 coating led to a slight decrease in this parameter for the sliced chicken sausage after 72 h of storage and to an increase after 144 h (Table 1).

Table 1. The dry mass of sliced chicken sausage.

Time [h]	Dry Mass [%]		
	C	M1	M2
0	66.8 ± 0.017	66.8 ± 0.017	66.8 ± 0.017
72	68.75 ± 0.004	69.66 ± 0.005	66.51 ± 0.009
144	69.40 ± 0.015	67.31 ± 0.001	68.31 ± 0.004

3.5. L a* b* Analysis*

It was demonstrated in the current work that the packaging material had an influence on ΔE_{lab} of the samples (Table 2). It was shown that ΔE_{lab} of sliced chicken sausage slices that were introduced into the bags without active coatings for 72 h was lower than sausage introduced into the bags covered with M1 and M2 active layers. In contrast to films coated with a layer containing the m2 mixture of *S. baicalensis* and *Glycyrrhiza* L. extracts, ΔE_{lab} for slices stored in bags coated with a layer containing M1 mixture was higher. Similar findings were noted after 144 h storage. The highest ΔE_{lab} was observed for samples stored in bags coated with active layers. The lowest ΔE_{lab} was noted in the control sample. It should be also mentioned that ΔE_{lab} was dependent on the packaging material. An analysis of sliced chicken sausage stored for 144 h found that a slightly higher ΔE_{lab} value was observed for samples introduced into bags with the M1 covering than into bags with the M2 coating.

Table 2. The ΔE_{lab} of sliced chicken sausage.

Time (h)		C	M1	M2
ΔE_{lab}	0	10.91 ± 0.87	10.91 ± 0.87	10.91 ± 0.87
ΔL		7.94 ± 1.14	7.94 ± 1.14	7.94 ± 1.14
ΔE_{lab}	72	10.43 ± 0.82	11.33 ± 0.44	10.80 ± 0.91
ΔL		7.60 ± 1.06	8.25 ± 0.57	7.86 ± 1.19
ΔE_{lab}	144	10.86 ± 0.28	11.13 ± 0.24	11.07 ± 0.25
ΔL		7.91 ± 0.37	8.10 ± 0.32	8.10 ± 0.52

4. Discussion

Freshness is an important property in the estimation of sliced chicken sausage quality, as this characteristic is directly linked to texture, microbial purity and perception of taste for consumers. Sliced sausages, packaged under vacuum, are cooked at 72–85 °C and may be stored at 1–8 °C for up to 30 days [2]. Chicken sausages packaged in a modified atmosphere can be stored at 4 °C or 10 °C up to 28 days. However, those chicken sausages packaged in modified atmosphere with biopreservative cultures, such as *Lactobacillus sakei* (B-2) and *Lactobacillus curvatus* (B-LC-48), inside the packaging may be stored for 42 days at 10 °C and 60 days at 4 °C with no spoilage [31]. Generally, chicken sausages are processed products containing a large amount of water. In consequence, they are highly sensitive to microorganisms that cause spoilage due to secondary contamination. As mentioned earlier, contamination during handling, cutting and packaging has a higher influence on the reduction of the shelf-life of meat samples than any initial contamination. In order to improve the microbial purity of ready-to-eat meat sausages, preservatives, e.g., sodium benzoate and potassium sorbate, may be added to reduce the bacterial load. However, these additives can cause changes in the texture and structure of meat samples and even the possibility of chemical substances remaining on meat products surfaces that may lead to health issues for consumers [2]. Active packaging materials, such as coated films or paper, for short time storage can be the ideal solution to this problem. These materials should be covered with coatings containing natural substances, such as plant extracts, which act as antimicrobial compounds [10,22]. The results of the current work showed that MHPC coating containing 50% of the *S. baicalensis** and *Glycyrrhiza* L. extracts (in m1—1*:2 ratio) mixture inhibited the growth of mesophilic bacteria for sliced chicken sausages that were stored for 72 h and decreased their number after 144 h of storage. It should be emphasised that no coliform bacteria—*L. monocytogenes*, *S. aureus*, nor *Salmonella* sp.—cells were detected in the chicken sausage slices after 72 h and 144 h of storage in PE bags covered with the M1 coating, confirming that these samples were acceptable for consumption even after 144 h of storage. It might be assumed that packaging covered with M1 coating, even if not recommended for the long-term storage of sliced chicken sausage, can be used for short-term safe keeping. These findings confirmed that the number of mesophilic microorganisms from ready-to-eat sliced sausage samples purchased from a local butcher ("0" sample—before storage) was high, proving that the sausages must have been contaminated during

slicing. It might be possible to predict and even suggest that if the number of bacteria of the "0" sample was lower, the sliced sausages could have been stored for longer than 144 h. Many consumers buy ready-to-eat meat products which are sliced by shop assistants and, in this case, the food products may be contaminated during slicing. This is why a packaging material, such as PE film, biopolymer film or paper covered in M1 coating, could be used to preserve sliced sausages during transport and short-term storage (72 h). The results obtained here were confirmed by Shiji et al. [6], who used an active packaging material to pack chicken sausages for short-term storage. As a control pack, polythene pouches were used by the authors. Biodegradable PVA-montmorillonite K10 clay nanocomposite blend containing silver nanoparticles as antimicrobial compounds were used as active pouches. The authors verified that after 4 days of storage at 4 °C, the sausage samples in the polythene pouches showed more bacterial growth than when compared to active pouches. The results of the present research determined that uncoated PE bags were not efficient enough to preserve sliced chicken sausages against microbial spoilage even after 3 days of storage. Shiji et al. [6] indicated that active nanocomposite film pouches strongly inhibited bacterial growth in the sausage samples after 4 days of storage. The results of this research proved that M1 coating preserved sausage samples even after 6 days. The active films containing 30% of green tea extract as an active compound were cut into circles and put on two surfaces of sausage pieces by Shahrampour et al. [32]. The active spacers and vacuum packaging were used for sausage samples in long-term storage (one month) at 4 °C. The sausage samples stored without coated films were used as control samples. The authors' results demonstrated that the growth of mesophilic microorganisms in uncoated control sausage samples were found to be higher than the total count of samples which were stored in the packaging containing active films, proving that active packaging had an influence on an improvement in the quality of the analysed wrapped food product samples. The results of the presented work also indicated that MHPC coating with 50% of m2 mixture (M2) had a small effect on the total bacterial count for the sausages that were stored for 72 h and was not effective against the bacteria for samples that were stored for 144 h. Similar results were obtained by Rüegg et al. [33], who applied polyethylene terephthalate (PET) films covered with modified calcium carbonate (MCC) coatings containing 30% thyme essential oil and 30% rosemary essential oil. They introduced a sliced cooked chicken breast into active bags and into uncoated bags for 6 days' storage. The results obtained by them authenticated that active coatings decreased microbial load compared to the uncoated films, though only slightly. It was proven that the modified atmosphere had a greater influence on microbial load than the active coatings. The influence of polyamide 6 composite films with silver–zinc crystals (SZ) powder used as sausage casings on the quality of the chicken sausages during storage (50 days, 4 °C and 12 °C, 75%–85% RH) was evaluated by Patiño et al. [34]. The microbiological results obtained by these authors proved that active casings containing SZ powder, in chicken sausages, were generally favoured. They showed that the counts of *S. aureus* (100 CFU/g) and coliforms exhibited minimal growth. Additionally, *Salmonella* spp. cells were not detected by the authors. To summarize, the inclusion of SZ reduced the growth of mesophilic aerobes during storage but had no impact on the shelf-life of the products.

One very important parameter that determines the quality of sliced chicken sausages is their texture. Products that are not cohesive enough or are too soft may create doubts in the consumer as to their quality. Cohesiveness is a highly important parameter that represents forces holding the food product together. Alternatively, tough, gummy or stringy sausages cannot be acceptable to consumers, as these products present excessively strong and non-specific resistance during mastication [35]. The findings indicated that the textural parameter of sliced chicken sausage after 144 h of storage in relation to cohesiveness increased for all of the described bags (when compared to the "0" sample) after 144 h of storage. It is tempting to suggest that the storage of sausage samples in uncoated PE bags and in the active bags improved the texture of the sausages. However, these results were not backed up by Patiño et al. [34] in their findings. These authors proved that

cohesiveness tended to decrease during storage. The authors suggested that a decrease in cohesiveness could be due to alterations in the interaction of fats and proteins with the rest of the components, affecting the structural parameters of the sausages. Araújo et al. [36] showed that to increase the cohesiveness of chicken sausages during storage, a commercial hydrolysed collagen powder or collagen gel extracted from chicken feet should be added. The present results showed that the springiness of sausage slices decreased for all described packaging materials (when compared to the "0" sample) except in the samples that were stored for 144 h in uncoated PE bags and this is a clear disadvantage.

The current work demonstrated that, unfortunately gumminess and chewiness increased after storage and this is considered a clear disadvantage. Analysing the samples that were stored for 72 h, it was observed that the best findings were obtained for sausage slices stored in bags covered with an M1 coating containing the m1 mixture, which is when the smallest increase of gumminess and chewiness in these samples was noted. This was proven in a microbial analysis which determined that the active packaging coated with the M1 layer was the best material for the short-term (72 h) storage of the ready-to-eat sliced chicken sausage. It is also worth considering the disadvantages of the PE films with an M1 coating, it was shown that water loss in sliced chicken sausage from these bags was the highest. The high water loss may explain the antimicrobial activity of the M1 layer. The water could cause the release of antimicrobial compounds from the coating and thus could contribute to the improvement of its activity. The higher increase of gumminess and chewiness was noted for the samples stored in bags covered with M2 coating after 72 h of storage. At the same time, water loss was not noted.

Contradictory results were obtained for the samples stored for 144 h. A microbial analysis determined that the active bags coated with the M1 layer were the best packaging for the 144 h time of storage of the sliced chicken sausage, because the total count was the lowest for these samples. It is also worth considering the advantage of the bags with M1 covering: it was demonstrated that water loss in sliced chicken sausage from this packaging was the lowest. However, textural parameter analysis did not corroborate these results. Unfortunately, it was noted that the best results were obtained for samples that were stored in uncoated bags, where the smallest increase of gumminess and chewiness was seen. An increase in these two parameters was observed for the sausages which were stored for 144 h in bags covered with the M2 layer but, conversely, in the case of 72 h storage and 144 h storage this caused water loss. In this case, the water loss had a clear influence on the increase of gumminess and chewiness. As Zeraatpisheh et al. [2] underlined, when moisture decreases, the meat proteins converge due to the formation of new cross-links; therefore, the gumminess and chewiness of the samples are elevated. Gumminess is considered a secondary factor and indicates the amount of energy needed to break down the food so that it is ready for swallowing. An increase in this parameter, as well as an increase in chewiness presented in this study, is a clear disadvantage because gumminess is associated with food hardness. Thus, in chicken sausages with increased gumminess and chewiness after storage, sausage slice hardness and difficulty in swallowing for the consumer will also be noticeable [2].

The results presented here showed that the highest ΔE_{lab} values were observed for sliced sausage slices introduced into bags with M1 active coating after 72 h of storage. It was also noticed that the highest ΔL values were observed for these samples. This means that the sliced chicken sausages taken from this packaging were the lightest. These findings were corroborated by Zhou et al. [37], who used curdlan film with the addition of bacterial cellulose and cinnamon essential oil as active compounds to obtain an active packaging material to preserve chicken meat. These authors also observed that L* values increased after 3 days' storage. The authors showed that this parameter decreased from the 3rd day to the 12th day of storage. Quite contradictory findings were described by Azlin-Hasim et al. [38], who noted that L* values increased in chicken meat after 6 and 12 days of storage in active LDPE films containing Ag nanoparticles as active agents. It may be concluded [37] that the released water from chicken meat enhanced the light reflection on

the chicken surface. However, the reduction of the L* value could probably be attributed to the oxidative discoloration of myoglobin in chicken. Additionally, another reason could be the complex biological changes, including pigment degradation caused by microorganisms. Alternatively, Patiño et al. [34] stated that, in the case of meat sausages, colour changes may be caused by chemical reactions between sodium nitrite and proteins, which can also be caused by any additives used in their formulation.

5. Conclusions

The results of the work demonstrated that the number of mesophilic microorganisms from ready-to-eat sliced sausage samples purchased from a local butcher ("0" sample—before storage) was high, confirming that the sausages must have been contaminated during slicing. It was shown that PE bags and spacers covered with M1 coating inhibited the growth of mesophilic bacteria for sliced chicken sausages that were stored for 72 h and decreased their number after 144 h of storage. It should be underlined that no coliform bacteria—*L. monocytogenes*, *S. aureus*, nor *Salmonella* sp.—cells were detected in the chicken samples after 72 h and 144 h of storage in PE packaging covered with the M1 coating, proving that these samples were acceptable for consumption. The textural analysis demonstrated that bags covered with M1 coating were the best packaging for 72 h of storage but not for 144 h storage.

To summarize, the bags and spacers covered with the coating containing 50% of the m1 mixture of *S. baicalensis* and *Glycyrrhiza* L. extracts were found to be the best packaging material for ready-to-eat chicken sausages which were purchased from a local butcher's shop. This active packaging could be used to preserve and maintain the quality and freshness of sausages samples, sliced by shop assistants after short-term storage (72 h) at 5 °C.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/coatings13040795/s1, Figure S1: The PE film and PE film covered with the active coatings (in 500× and 2000× magnifications).

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